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15 WORLD CONGRESS OF GYNECOLOGICAL ENDOCRINOLOGY

III CONGRESSO ISGE ITALIA

Firenze, 7-10 marzo 2012

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1

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Contents

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FORMA FARMACEUTICA** - Compressa rivestita: compresse rivestite di zucchero, di colore bianco, arrotondate, biconvesse senza impressioni su entrambi i lati. - **4. INFORMAZIONI CLINICHE. 4.1.Indicazioni terapeutiche.** Contraccezione orale. **4.2.Posologia e modo di somministrazione.** Come assumere GESTODIOL. Le compresse devono essere assunte nell'ordine indicato sulla confezione ogni giorno approssimativamente alla stessa ora. Una compressa al giorno per 21 giorni. Ogni confezione successiva deve essere iniziata dopo un intervallo di 7 giorni in cui non verrà assunta alcuna compressa: durante questo lasso di tempo si verificherà un'emorragia da sospensione. Quest'emorragia inizia solitamente il secondo o terzo giorno dopo aver assunto l'ultima compressa e potrebbe continuare anche dopo l'inizio della confezione successiva. Come cominciare ad assumere GESTODIOL. Nel caso in cui non ci sia stato alcun trattamento contraccettivo ormonale nel mese precedente. È necessario assumere la prima compressa il primo giorno del ciclo naturale della donna (vale a dire il primo giorno del suo ciclo mestruale). È possibile cominciare ad assumere le pillole dal secondo al quinto giorno ma in questi casi si raccomanda di usare anche un metodo contraccettivo di barriera per i primi sette giorni d' assunzione delle compresse durante il primo ciclo. In caso di passaggio da un'altra pillola contraccettiva orale di tipo combinato. La donna deve cominciare ad assumere GESTODIOL il giorno dopo l'ultima compressa attiva del suo precedente contraccettivo – ma non più tardi del giorno successivo al completamento dell'usuale periodo in cui non assume alcuna pillola oppure assume placebo come previsto dal farmaco contraccettivo precedente. Quando si passa da un contraccettivo solo progestinico (pillola solo al progesterone (mini-pillola, iniezione, impianto)oppure da un sistema intrauterino a rilascio di ormone progestinico (IUS). La donna può effettuare il passaggio dalla pillola solo al progesterone (POP) in qualsiasi momento del ciclo. La prima compressa deve essere assunta il giorno dopo aver assunto una qualsiasi delle compresse nella confezione di POP. Nel caso di un impianto o di una IUS l'assunzione di GESTODIOL deve cominciare lo stesso giorno nel quale l'impianto viene rimosso. Nel caso di un iniettabile, GESTODIOL deve essere iniziato nel giorno in cui dovrebbe essere praticata la successiva iniezione. In tutti questi casi si raccomanda alla donna di usare anche un metodo contraccettivo di barriera per i primi sette giorni di assunzione delle pillole. Dopo un aborto al primo trimestre. La donna può iniziare immediatamente a prendere le pillole. Se si attiene a queste istruzioni non sono necessarie ulteriori misure contraccettive. Dopo un parto o un aborto al secondo trimestre. Per l'uso in donne che allattano si veda il paragrafo 4.6. Si raccomanda alla donna di iniziare a prendere le compresse al 21°-28° giorno dopo il parto, se non allatta al seno, o dopo un aborto al secondo trimestre. Se inizia più tardi, la donna deve essere avvertita di usare anche un metodo contraccettivo di barriera per i primi sette giorni di assunzione delle pillole. Se nel frattempo si fossero avuti rapporti sessuali, prima di iniziare effettivamente l'assunzione delle pillole si deve escludere una gravidanza oppure la donna deve attendere la comparsa della sua prima mestruazione. Mancata assunzione di compresse. La mancata assunzione di una compressa entro 12 ore dall'ora consueta non pregiudica la protezione contraccettiva. La donna deve prendere la compressa appena se ne ricorda e continuare ad assumere il resto delle compresse come al solito. La mancata assunzione di una compressa per più di 12 ore dall'ora consueta può diminuire la protezione contraccettiva. Le due regole seguenti possono essere utili nella gestione della mancata assunzione di compresse. 1. l'assunzione delle compresse non deve mai essere sospesa per periodi superiori ai 7 giorni. 2. Servono 7 giorni di ingestione ininterrotta di compresse per ottenere una sufficiente soppressione dell'asse ipotalamo-pituitario-gonadale. Pertanto il consiglio che segue può essere dato nella pratica giornaliera: **Settimana 1.** La donna deve prendere l'ultima compressa dimenticata non appena se ne ricorda, anche se questo significa che deve assumere 2 compresse contemporaneamente. Dopodiché deve continuare ad assumere le compresse alla solita ora. Contemporaneamente deve usare un metodo di barriera, ad es. un preservativo, per i successivi 7 giorni. Se nei 7 giorni precedenti si sono avuti rapporti sessuali la donna deve tenere in considerazione la possibilità di poter essere incinta. Tante più compresse sono state dimenticate e tanto più ciò è avvenuto in prossimità del periodo del mese in cui le compresse non vengono assunte, tanto maggiore è il rischio che si instauri una gravidanza. **Settimana 2.** La donna deve prendere l'ultima compressa dimenticata non appena se ne ricorda, anche se questo significa che deve assumere 2 compresse contemporaneamente. Dopodiché deve continuare ad assumere le compresse alla solita ora. Se le compresse sono state assunte correttamente per 7 giorni prima della dimenticanza non è necessario prendere ulteriori precauzioni contraccettive. In caso contrario o se sono state dimenticate più compresse la donna deve comunque usare un metodo di barriera, ad es. un preservativo, per i successivi 7 giorni. **Settimana 3.** Dato l'avvicinarsi del periodo di sospensione il rischio di una ridotta protezione anticoncezionale è maggiore. È comunque possibile prevenire la riduzione della protezione anticoncezionale regolando l'assunzione delle compresse. Attenendosi a una qualunque delle due opzioni seguenti non è pertanto necessario prendere alcuna precauzione contraccettiva supplementare, fatto salvo che le compresse siano state assunte correttamente per 7 giorni prima della dimenticanza. In caso contrario è opportuno consigliare alla donna di seguire la prima delle due opzioni e di usare allo stesso tempo un metodo di barriera, ad es. un preservativo, per i 7 giorni successivi. **1.** La donna deve prendere l'ultima compressa dimenticata al più presto, anche se questo significa che deve assumere 2 compresse contemporaneamente. Dopodiché deve continuare ad assumere le compresse alla solita ora. Incomincerà la nuova confezione immediatamente dopo aver assunto l'ultima compressa della confezione in uso; in questo caso non vi sarà il periodo di sospensione tra le confezioni. È improbabile che si verifichino le mestruazioni fino al termine della seconda confezione di compresse, tuttavia si potrebbe notare emorragia intermestruale o metrorragia durante l'assunzione delle compresse. **2.** È possibile che alla donna venga suggerito di sospendere l'assunzione delle compresse dalla confezione in uso. In qual caso si avrà un periodo di sospensione della durata massima di 7 giorni, inclusi i giorni in cui la compressa è stata dimenticata, dopodiché la donna inizierà una nuova confezione. Se, dopo che la donna ha dimenticato di assumere delle compresse, non si presentano le mestruazioni nel primo usuale intervallo libero da pillola, si deve considerare la possibilità che la donna sia incinta. Cosa fare in caso di vomito/diarrea. Se si manifesta vomito entro 3-4 ore dall'assunzione di una compressa, quest'ultima potrebbe non venire completamente assorbita. In questo caso ci si attenga alle istruzioni sopra indicate inerenti le compresse dimenticate. A meno che la diarrea non sia estremamente grave, essa non influisce sull'assorbimento dei contraccettivi orali combinarti, per cui non è necessario ricorrere a metodi contraccettivi supplementari. Se la diarrea grave perdura per 2 o più giorni ci si attenga alle procedure previste per le pillole dimenticate. Se la donna non desidera variare la consueta assunzione di compresse, deve prendere una compressa (o compresse) extra da un'altra confezione. Come spostare o ritardare il mestruo. Per ritardare il mestruo, la donna dovrà continuare l'assunzione di GESTODIOL passando da una confezione blister ad un'altra, senza periodo di sospensione. Il mestruo può essere ritardato per quanto si desidera ma non oltre la fine della seconda confezione. Quando si ritarda il mestruo è possibile che si verifichino episodi di sanguinamento da sospensione o emorragia intermestruale. L'assunzione di GESTODIOL dovrà essere ripresa regolarmente al termine del consueto intervallo in cui non viene assuna alcuna compressa. Per spostare il mestruo ad un giorno nella settimana diverso rispetto a quello previsto con le attuali compresse, si può consigliare alla donna di abbreviare il successivo intervallo libero da pillola di quanti giorni lei desidera. Più breve è questo intervallo e maggiore sarà il rischio di non avere sanguinamento mestruale ma metrorragia e emorragia intermestruale durante l'assunzione delle compresse della confezione successiva (questo si verifica anche quando si ritarda il mestruo). **4.3. Controindicazioni.** I contraccettivi orali combinati (COC) non devono essere usati se una delle condizioni sotto indicate è presente. Se una tale condizione si dovesse manifestare per la prima volta durante l'impiego dei COC il loro uso deve essere immediatamente sospeso. - • Patologia tromboembolica venosa in fase attiva o in anamnesi (trombosi venosa profonda, embolia polmonare). • Tromboembolia arteriosa in fase attiva o in anamnesi (infarto del miocardio, patologie cerebrovascolari) oppure sintomi prodromici (angina pectoris e attacco ischemico transitorio) (vedi paragrafo 4.4). • Predisposizione ereditaria o acquisita alla trombosi venosa o arteriosa come carenza di antitrombina, carenza di proteina C, carenza di proteina S, resistenza alla proteina C attivata (APC), anticorpi antifosfolipidi (anticorpi anticardiolipina, lupus anticoagulante), iperomocisteinemia. • Fattori di rischio multipli o consideревoli per la trombosi arteriosa (vedi paragrafo 4.4). • Grave ipertensione. • Diabete complicato da micro- o macroangiopatia. • Grave dislipoproteinemia. • Noti o sospetti tumori maligni ormono-dipendenti (ad es. a carico degli organi genitali o della mammella). • Grave patologia epatica concomitante o in anamnesi fintanto che i valori di funzionalità epatica non sono rientrati nella normalità. • Tumori epatici benigni o maligni concomitanti o in anamnesi. • Sanguinamento vaginale di natura non accertata. • Emicrania con sintomatologia neurologica focale. • Ipersensibilità ai principi attivi o ad uno qualsiasi degli eccipienti. **4.4.Avvertenze speciali e precauzioni d'impiego.** Valutazione ed esame prima di iniziare l'assunzione dei contraccettivi orali combinati. Prima dell'inizio o della ripresa del trattamento con contraccettivi orali combinati è necessario che il medico analizzi l'anamnesi personale e familiare della paziente e che venga esclusa una gravidanza. Sulla base delle controindicazioni (vedi paragrafo 4.3) e delle avvertenze (vedi "Avvertenze" in questa sezione) è necessario misurare la pressione sanguigna e sottoporre la paziente ad un esame fisico, se clinicamente indicato. Alla donna viene richiesto di leggere attentamente il foglio illustrativo e di attenersi alle istruzioni fornite. La frequenza e la natura di ulteriori controlli periodici devono basarsi su linee guida di pratica stabilita ed essere adattate alla singola donna. Avvertenze. In generale. Informare le donne che i contraccettivi ormonali non proteggono dall'HIV (AIDS) o da altre infezioni sessualmente trasmessibili. Se uno qualunque dei fattori di rischio sotto menzionati è presente, valutare caso per caso i benefici connessi all'uso del COC con i possibili rischi per ogni singola donna e discuterne con la donna prima di cominciare l'assunzione del contraccettivo orale combinato. In caso di aggravamento, esacerbazione o insorgenza di una qualsiasi di queste condizioni o fattori di rischio è opportuno che la donna prenda contatto con il suo medico. Il medico deciderà se interrompere l'assunzione del COC. 1.Disturbi della circolazione. L'uso di qualsiasi COC aumenta il rischio di tromboembolia venosa (TEV) rispetto al non uso. L'eccesso di rischio di TEV è massimo durante il primo anno in cui una donna fa uso di un COC per la prima volta. L'aumento di rischio è inferiore rispetto al rischio di TEV associato alla gravidanza, che è stimato in 60 casi ogni 100.000 gravidanze. La TEV risulta fatale nell' 1-2% dei casi. In diversi studi epidemiologici è stato riscontrato che nelle donne che usano contraccettivi orali combinati contenenti etinilestradiolo, per lo più alla dose di 30 µg, e un progestinico come gestodene il rischio di TEV è aumentato rispetto alle donne che usano contraccettivi orali combinati contenenti meno di 50 µg di etinilestradiolo ed il progestinico levonorgestrel. Relativamente ai contraccettivi orali combinati contenenti 30 µg di etinilestradiolo in combinazione con desogestrel o gestodene in confronto a quelli contenenti meno di 50 µg di etinilestradiolo e levonorgestrel, è stato stimato che il rischio relativo complessivo di TEV è compreso tra 1,5 e 2,0. Nel caso di contraccettivi orali combinati contenenti levonorgestrel con meno di 50 µg di etinilestradiolo l'incidenza di TEV è di circa 20 casi su ogni 100.000 anni-donna di utilizzo. Per quanto riguarda GESTODIOL l'incidenza varia da 30 a 40 casi per 100.000 anni-donna di utilizzo, vale a dire 10-20 casi aggiuntivi ogni 100.000 anni-donna di utilizzo. L'impatto del rischio relativo sul numero di casi addizionali sarebbe massimo in donne durante il primo anno di utilizzo del contraccettivo orale combinato quando il rischio di TEV con tutti i contraccettivi orali combinati è massimo. Molto raramente è stata segnalata trombosi in altri vasi sanguigni, vale a dire di tipo epatico, mesenterico, renale oppure a carico delle vene e delle arterie della retina in utilizzatrici di contraccettivi orali. Non vi è consenso circa la possibilità che l'insorgenza di questi casi sia correlata all'uso di COC. Il rischio che si sviluppi tromboembolia venosa aumenta: - • con l'avanzamento dell'età; • in caso di anamnesi familiare positiva (ad es. tromboembolia venosa che ha riguardato un parente o un consanguineo più soggetti di età relativamente giovane). In caso di sospetta predisposizione ereditaria, la donna deve essere indirizzata da uno specialista prima che le sia prescritto un contraccettivo orale; • in caso di obesità (indice di massa corporea superiore a 30 Kg/m2); • immobilizzazione prolungata, chirurgia maggiore, intervento chirurgico alle gambe o trauma maggiore. In questi casi è raccomandata la sospensione del trattamento con i contraccettivi orali (nel caso di un'operazione chirurgica programmata almeno 4 settimane prima) e non deve essere assunto fino a 2 settimane dopo la completa deambulazione; • non vi è consenso sul possibile ruolo di vene varicose e tromboflebiti superficiali nella tromboembolia venosa. In generale l'uso di COC è stato associato ad un aumento del rischio di infarto acuto del miocardio (AMI) o di ictus, rischio questo fortemente influenzato dalla presenza di altri fattori di rischio (ad es. fumo, pressione sanguigna alta ed età) (vedi anche sotto). Questi eventi si verificano raramente. Il rischio di eventi tromboembolici aumenta con: • l'avanzamento dell'età; • fumo (con forti fumatrici e con l'avanzare dell'età il rischio aumenta ulteriormente, soprattutto se si tratta di donne con più di 35 anni di età); • dislipoproteinemia; • obesità (indice di massa corporea superiore a 30 Kg/m2); •ipertensione; •valvulopatia cardiaca; • fibrillazione atriale; • anamnesi familiare positiva (ad es. trombosi arteriosa che ha riguardato un parente o un consanguineo di età relativamente giovane). Se si sospetta una predisposizione ereditaria la donna deve essere indirizzata da uno specialista prima che le sia prescritto un contraccettivo orale. Sintomi di trombosi venosa ed arteriosa possono includere: • dolore e/o gonfiore unilaterale ad una gamba; • improvviso grave dolore toracico, che può o meno estendersi al braccio sinistro; • fiato corto improvviso; • tosse improvvisa; • cefalea insolita, grave, prolungata; • improvvisa perdita parziale o completa della vista; • diplopia; • difficoltà nel parlare o afasia; • vertigini; • collasso accompagnato o meno da crisi epilettiche focali; • debolezza o improvviso intorpidimento molto marcato di un lato o una parte del corpo; • disturbi motori; • addome "acuto". Si deve tenere in considerazione l'aumento del rischio di tromboembolia venosa durante il puerperio. Altre condizioni mediche correlate ai disturbi vascolari sono: diabete mellito, lupus eritematoso sistemico, sindrome emolitico-uremica, malattia infiammatoria cronica intestinale (morbo di Crohn oppure colite ulcerosa) e anemia a cellule falciformi. Un aumento della frequenza e della gravità dell'emicrania (che può essere prodromica in caso di malattia cerebrovascolare) durante l'impiego di contraccettivi orali deve far prendere in considerazione l'immediata sospensione dei contraccettivi orali. Fra i parametri biochimici indicativi della predisposizione ereditaria o acquisita alla trombosi venosa o arteriosa vi sono: resistenza alla

proteina C attivata (APC), mutazione del fattore V di Leiden, iperomocisteinemia, carenza di antitrombina-III, carenza di proteina C, carenza di proteina S, anticorpi antifosfolipidi (anticorpi anticardiolipina, lupus anticoagulante). Mentre valuta il rapporto rischio/beneficio il medico deve tenere presente che il trattamento adeguato di una condizione può ridurre il rischio associato di trombosi e che il rischio associato alla gravidanza è maggiore rispetto a quello connesso all'uso di COC. 2. Tumori: Cancro della cervice. In alcuni studi epidemiologici si è riferito un rischio maggiore di cancro cervicale uterina nelle utilizzatrici a lungo termine dei COC ma non è ancora chiaro fino a che punto questo rilievo possa essere influenzato dagli effetti aggravanti del comportamento sessuale e di altri fattori quali il papilloma virus umano (HPV). Carcinoma della mammella. Una meta-analisi di 54 studi epidemiologici ha riferito un rischio relativo leggermente superiore (RR=1,24) di diagnosi di cancro della mammella fra le donne che attualmente usano COC. L'eccedenza di rischio scompare gradualmente nel corso dei 10 anni seguenti all'interruzione dell'uso dei COC. Poiché il cancro della mammella è raro nelle donne di meno di 40 anni, il numero superiore di diagnosi di tumore alla mammella fra le utilizzatrici attuali e recenti di COC è limitato in rapporto al rischio globale di cancro della mammella. Questi studi non forniscono evidenza di causalità. L'andamento superiore del rischio osservato potrebbe essere dovuto ad una diagnosi precoce del cancro della mammella nelle utilizzatrici di COC, agli effetti biologici dei COC o a una combinazione di entrambi i fattori. Il cancro alla mammella diagnosticato nelle donne che hanno usato COC tende ad essere meno avanzato dal punto di vista clinico rispetto alle forme tumorali riscontrate fra le donne che non hanno mai assunto COC. Tumori epatici. Tra le utilizzatrici di COC si sono riferiti tumori epatici benigni e maligni. In casi isolati questi tumori hanno portato ad emorragie intra-addominali ad esito potenzialmente fatale. Pertanto, considerare la possibilità di tumore epatico nella diagnosi differenziale, quando un'utilizzatrice di COC presenti severo dolore all'addome superiore, ingrossamento del fegato (epatomegalia) oppure segni di emorragia intra-addominale. 3. Altre condizioni. Le donne affette da ipertrigliceridemia, o anamnesi familiare della stessa, possono essere a rischio maggiore di pancreatite mentre usano COC. In caso di disturbi acuti o cronici della funzionalità epatica potrà essere necessaria l'interruzione di GESTODIOL, fino al ripristino ai valori normali dei marker della funzionalità epatica. Gli ormoni steroidei potrebbero essere scarsamente metabolizzati in pazienti con funzionalità epatica compromessa. Malgrado si siano riferiti piccoli innalzamenti della pressione arteriosa in molte donne che assumono contraccettivi orali combinati, gli innalzamenti clinicamente significativi sono rari. Se, durante l'assunzione di un contraccettivo ormonale combinato si sviluppa un'ipertensione clinica persistente bisogna sospendere l'assunzione del contraccettivo ormonale combinato e trattare l'ipertensione. L'assunzione del contraccettivo orale combinato potrà riprendere se risulta possibile ottenere valori normotensivi mediante la terapia Se il medico lo ritiene opportuno, l'uso della pillola può essere ripreso quando i valori della pressione rientreranno nella norma in seguito a terapia antiipertensiva. Sia con la gravidanza che con l'uso di COC possono comparire o peggiorare delle condizioni qui di seguito riportate. Tuttavia, le prove di un'associazione con l'uso dei COC non sono decisive: ittero e/o prurito associato a colestasi; sviluppo di calcoli biliari; porfiria; lupus eritematoso sistemico; sindrome emoliticouremica; corea di Sydenham; herpes gestationis; perdita di udito dovuta a otosclerosi. I contraccettivi orali combinati possono avere un effetto sulla resistenza periferica all'insulina e sulla tolleranza al glucosio. È pertanto necessario che le pazienti diabetiche vengano attentamente monitorate durante l'impiego dei COC. GESTODIOL contiene lattosio e saccarosio. Le pazienti con rari problemi ereditari di intolleranza al galattosio, deficit di Lapp-lattasi o malassorbimento di glucosio-galattosio oppure con rari problemi di intolleranza al fruttosio non devono assumere questo medicinale. Durante l'uso dei COC si è riferito l'aggravamento della depressione endogena, dell'epilessia (vedi paragrafo 4.5 Interazioni), del morbo di Crohn e della colite ulcerosa. È possibile che si manifesti cloasma, specialmente nelle utilizzatrici con anamnesi di cloasma gravidarum. Le donne con tendenza al cloasma devono evitare l'esposizione al sole o alla radiazione ultravioletta mentre assumono i COC. Le preparazioni erboristiche contenenti Iperico o erba di San Giovanni (Hypericum perforatum) non devono essere assunte contemporaneamente a GESTODIOL a causa del rischio di diminuzione delle concentrazioni plasmatiche e degli effetti clinici di GESTODIOL (vedi paragrafo 4.5). Efficacia ridotta. L'efficacia dei contraccettivi orali può essere ridotta nel caso in cui ci si dimentichi di assumere delle compresse, in presenza di diarrea grave o vomito (vedi paragrafo 4.2) oppure in caso di uso concomitante di altri medicinali (vedi paragrafo 4.5). Ciclo irregolare. Come con tutti i contraccettivi ormonali combinati, potrà verificarsi la perdita irregolare di sangue (emorragia intermestruale o metrorragia), particolarmente nei primi mesi di assunzione. Per questo motivo, un'opinione medica circa la perdita irregolare di sangue avrà utilità solo dopo un periodo di adattamento di tre cicli circa. Se la metrorragia persiste sarà necessario considerare la possibilità di usare COC con un contenuto ormonale più alto. Se la metrorragia si verifica dopo precedenti cicli regolari occorre considerare cause non di natura ormonale e prendere adeguate misure diagnostiche per escludere la presenza di una patologia maligna o di una gravidanza. Occasionalmente potrebbe non esservi alcuna emorragia da sospensione nell'intervallo in cui non vengono assunte le compresse. Se le compresse sono state assunte secondo le istruzioni di cui al paragrafo 4.2, è improbabile che la donna sia incinta. Tuttavia, se le compresse non sono state assunte in base a dette istruzioni precedentemente alla prima emorragia da sospensione saltata, oppure se la donna salta consecutivamente due emorragie da sospensione, è necessario escludere la gravidanza prima di proseguire l'assunzione del COC. **4.5. Interazioni con altri medicinali ed altre forme di interazione.** Le interazioni con medicinali in grado di portare ad una elevata clearance degli ormoni sessuali possono comportare metrorragia ed insuccesso della contraccezione orale. Questo effetto è stato stabilito nel caso di idantoine, barbiturici, primidone, carbamazepina e rifampicina, ed è risultato sospetto nel caso di oxcarbazepina, topiramato, griseofulvina, felbamato e ritonavir. Il meccanismo di queste interazioni sembra essere basato sulle proprietà di induzione degli enzimi epatici di questi medicinali. In generale la massima induzione enzimatica non si ha nelle prime 2-3 settimane dopo l'inizio del trattamento, ma l'effetto può essere sostenuto per almeno 4 settimane dopo l'interruzione della terapia. Si sono riferiti anche casi di insuccesso della contraccezione con antibiotici quali ampicillina e tetracicline. Il meccanismo di questo effetto non è stato chiarito. Le donne in trattamento a breve termine con uno qualsiasi dei gruppi di farmaci sopra citati o con singoli medicinali, devono usare temporaneamente un metodo di barriera oltre alla pillola anticoncezionale, ciò deve avvenire per tutto il tempo in cui questo medicinale viene assunto contemporaneamente alla pillola come pure nei sette giorni successivi alla sua sospensione. Le donne in trattamento con rifampicina devono usare un metodo di barriera contemporaneamente al contraccettivo orale durante tutto il periodo in cui assumono la rifampicina come pure nei 28 giorni successivi alla sua sospensione. Se la somministrazione concomitante del medicinale continua oltre il numero di compresse anticoncezionali nella confezione, la donna deve iniziare la confezione successiva, senza osservare il consueto intervallo di sospensione. Per le donne in terapia a lungo termine con induttori degli enzimi epatici, è necessario considerare un altro metodo contraccettivo. Le pazienti che assumono GESTODIOL non devono usare contemporaneamente preparazioni/prodotti medicinali alternativi contenenti Hypericum perforatum (Iperico o erba di San Giovanni) poiché essi potrebbero causare una perdita dell'effetto contraccettivo. Si sono riferite metrorragia e gravidanze indesiderate. L'Hypericum perforatum (Iperico o erba di San Giovanni) aumenta, mediante induzione enzimatica, la quantità di enzimi che metabolizzano i prodotti medicinali. L'effetto di induzione enzimatica potrebbe persistere per almeno 1-2 settimane dalla cessazione del trattamento con Hypericum. Effetti dei contraccettivi orali combinati su altri farmaci: i contraccettivi orali possono interferire con il metabolismo di altri farmaci. Ne può conseguire un aumento (ad es. ciclosporina) o una diminuzione (lamotrigina)delle concentrazioni plasmatiche e tissutali. Test di laboratorio. L'impiego di steroidi contraccettivi può influenzare i risultati di alcuni esami di laboratorio tra cui i parametri biochimici della funzionalità epatica, tiroidea, corticosurrenalica e renale, i livelli plasmatici delle proteine (di trasporto), per esempio della globulina legante i corticosteroidi e delle frazioni lipido/lipoproteiche, i parametri del metabolismo dei carboidrati ed i parametri della coagulazione e della fibrinolisi. Le variazioni rientrano, in genere, nei limiti dei valori normali di laboratorio. **4.6 Gravidanza ed allattamento.** GESTODIOL è controindicato durante la gravidanza. In caso di gravidanza durante l'assunzione di GESTODIOL sospendere immediatamente il trattamento. Estesi studi epidemiologici non hanno evidenziato né un aumento del rischio di difetti congeniti in bambini nati da donne che hanno assunto contraccettivi orali combinati prima della gravidanza, né effetti teratogeni a seguito di involontaria assunzione di contraccettivi orali combinati durante la gravidanza. L'allattamento può essere influenzato dagli steroidi contraccettivi in quanto essi possono ridurre il volume ed alterare la composizione del latte materno. Piccole quantità di steroidi contraccettivi e/o di loro metaboliti possono essere escreti nel latte materno. Pertanto, l'uso di steroidi contraccettivi non è in genere raccomandato in madri che allattano fino al termine del completo svezzamento. **4.7 Effetti sulla capacità di guidare veicoli e sull'uso di macchinari.** GESTODIOL non ha effetti, se non minimi, sulla capacità di guidare veicoli e di usare macchinari. **4.8 Effetti indesiderati.** Gli eventi avversi riferiti con maggior frequenza (>1/10) sono sanguinamento irregolare, nausea, aumento ponderale, tensione mammaria e cefalea. Essi si manifestano solitamente all'inizio della terapia e sono transitori.

Classificazione sistemica organica	Comune (da = 1/100 a <1/10)	Non comune (da = 1/1000 a <1/100)	Raro (da = 1/10000 a <1000)	Molto raro (<1/10000)
Patologie del sistema nervoso	Cefalea			
Nervosismo			Corea	
Patologie dell'occhio	Irritazione oculare quando si portano lenti a contatto			
Disturbi della vista				
Patologie dell'orecchio e del labirinto			Otosclerosi	
Patologie gastrointestinali	Nausea	Vomito	Colelitiasi	Pancreatite
Patologie della cute e del tessuto sottocutaneo	Acne		Cloasma	
Disordini del metabolismo e della nutrizione		Iperlipidemia		
Patologie vascolari	Emicrania	Ipertensione	Tromboembolia venosa	
Eventi tromboembolici arteriosi				
Patologie sistemiche e condizioni relative alla sede di somministrazione	Aumento ponderale			
Ritenzione idrica				
Disturbi del sistema immunitario			Lupus eritematoso	
Patologie dell'apparato riproduttivo e della mammella	Sanguinamento irregolare			
Amenorrea				
Ipomenorrea				
Tensione mammaria		Alterata secrezione vaginale		
Disturbi psichiatrici	Alterazioni della libido			
Depressione				
Irritabilità				

I seguenti gravi effetti indesiderati sono stati riportati in donne che assumono COC, vedi paragrafi 4.3 e 4.4. • Tromboembolia venosa, vale a dire trombosi venosa profonda in una gamba o alle pelvi ed embolia polmonare. • Eventi tromboembolici arteriosi. • Tumori epatici. • Patologia della cute e del tessuto sottocutaneo: cloasma. La frequenza di diagnosi di cancro della mammella fra le donne che assumono COC è leggermente maggiore. Poiché il cancro della mammella è raro nelle donne con meno di 40 anni. Il numero superiore è limitato in rapporto al rischio globale di cancro alla mammella. Non è noto il rapporto di causalità con i COC. Per ulteriori informazioni vedere i paragrafi 4.3 e 4.4. **4.9. Sovradosaggio.** Non sono stati riferiti effetti indesiderati seri in seguito a sovradosaggio. I sintomi che possono manifestarsi in seguito ad sovradosaggio sono: nausea, vomito e sanguinamento vaginale. Non c'è antidoto, e il trattamento deve essere sintomatico. - **5. PROPRIETÀ FARMACOLOGICHE. 5.1. Proprietà farmacodinamiche.** Categoria farmacoterapeutica: Contraccettivi ormonali per uso sistemico. Codice ATC: G03AA10. L'effetto contraccettivo delle pillole anticoncezionali si basa sull'interazione di vari fattori, i più importanti dei quali sono l'inibizione dell'ovulazione e le modifiche dell'endometrio. Oltre a prevenire il concepimento i COC possiedono diverse caratteristiche positive che, accanto alle proprietà negative (illustrate al paragrafo 4.8 Avvertenze, Effetti indesiderati), possono aiutare nella scelta del metodo da adottare per il controllo delle nascite. Il ciclo mestruale è più regolare e le mestruazioni stesse sono spesso meno dolorose ed il sanguinamento più leggero. Quest'ultimo aspetto può determinare una diminuzione dei casi di carenza di ferro. **5.2. Proprietà farmacocinetiche.** Gestodene. Assorbimento. Dopo somministrazione orale il gestodene viene rapidamente e completamente assorbito. Dopo somministrazione di una dose singola la massima concentrazione sierica di 4 ng/ml viene raggiunta dopo circa un'ora. La biodisponibilità è intorno al 99%. Distribuzione. Gestodene è legato all'albumina sierica ed alle globuline leganti gli ormoni sessuali (SHBG). Solo l'1-2% del gestodene totale in siero viene ritrovato come steroide libero, mentre il 50-70% è specificamente legato alle SHBG. L'aumento delle SHBG indotto dall'etinilestradiolo influenza la distribuzione delle proteine sieriche con conseguente aumento della frazione legata alle SHBG e diminuzione della frazione legata all'albumina. Il volume di distribuzione apparente del gestodene è di 0,7 l/kg. Metabolismo. Il gestodene viene completamente metabolizzato tramite i noti canali del metabolismo degli steroidi. L'entità della clearance metabolica dal siero è pari a 0,8 ml/min/kg. Non si manifestano interazioni quando il gestodene viene assunto insieme all'etinilestradiolo. Eliminazione. I livelli sierici del gestodene diminuiscono in modo bifasico. La fase di eliminazione terminale è caratterizzata da un'emivita di 12-15 ore. Il gestodene non viene escreto immodificato. I suoi metaboliti vengono escreti nelle urine e nella bile in un rapporto di 6:4. L'emivita di escrezione dei metaboliti è pari a circa 1 giorno. Steady-state. La farmacocinetica del gestodene è influenzata dai livelli sierici di SHBG che aumentano di tre volte con l'etinilestradioloo. In seguito all'assunzione giornaliera i livelli sierici di gestodene aumentano di circa quattro volte il valore della dose singola e raggiungono lo steady-state entro la seconda metà del ciclo di trattamento. Etinilestradiolo. Assorbimento. Dopo somministrazione orale l'etinilestradiolo viene rapidamente e completamente assorbito. Il picco dei livelli plasmatici, pari a circa 80 pg/ml, viene raggiunto in 1-2 ore. La biodisponibilità assoluta, dopo coniugazione presistemica e metabolismo di primo passaggio, è all'incirca del 60%. Distribuzione. Durante l'allattamento lo 0,02% della dose giornaliera della madre passa nel latte. L'etinilestradiolo è largamente, ma non specificamente, legato all'albumina (approssimativamente per il 98,5%) e induce un aumento nelle concentrazioni sieriche dell'SHBG. È stato determinato un volume di distribuzione apparente di circa 5 l/kg. Metabolismo. L'etinilestradiolo è soggetto a coniugazione presistemica a livello sia della mucosa dell'intestino tenue sia del fegato. La principale via metabolica dell'etinilestradiolo è l'idrossilazione aromatica ma si forma anche una ampia varietà di metaboliti idrossilati e metilati, presenti come metaboliti liberi e coniugati con glucuronidi e solfati. L'entità della clearance metabolica è pari a circa 5 ml/min/kg. Eliminazione. I livelli sierici dell'etinilestradiolo diminuiscono in modo bifasico, con una fase di eliminazione terminale con un'emivita di circa 24 ore. L'etinilestradiolo immodificato non viene escreto, ma i suoi metaboliti sono escreti in un rapporto urina:bile pari a 4:6. L'emivita dell'escrezione dei metaboliti è di circa 1 giorno. Steady-state. Le concentrazioni allo steady-state vengono raggiunte dopo 3-4 giorni ed i livelli sierici dell'etinilestradiolo sono più elevati del 30-40% rispetto alla singola assunzione. **5.3. Dati preclinici di sicurezza.** Etinilestradiolo e gestodene non sono genotossici. Gli studi di carcinogenicità con etinilestradiolo da solo o in associazione con vari progestinici non mostrano alcun pericolo carcinogenico in donne che usano il farmaco come contraccettivo come indicato. È tuttavia necessario tenere presente che gli ormoni sessuali possono promuovere la crescita di alcuni tessuti e tumori ormono-dipendenti. Studi di tossicità riproduttiva su fertilità, sviluppo fetale o performance riproduttiva condotti con etinilestradiolo da solo o in associazione con progestinici non hanno fornito indicazioni di un rischio di effetti avversi nell'uomo conseguenti all'impiego del preparato secondo quanto raccomandato. - **6. INFORMAZIONI FARMACEUTICHE. 6.1.Elenco degli eccipienti.** Nucleo della compressa: Magnesio stearato, Povidone K-25, Amido di mais, Lattosio monoidrato. Rivestimento della compressa: Povidone K-90, Macrogol 6000, Talco, Calcio carbonato, Saccarosio, Cera di lignite. **6.2 Incompatibilità.** Non pertinente. **6.3. Periodo di validità.** Tre anni. **6.4. Speciali precauzioni per la conservazione.** Non conservare a temperatura superiore a 30°C. **6.5. Natura e contenuto del contenitore.** Blister: PVC/Alluminio. Confezioni: 1 X 21 compresse; 3 X 21 compresse; 6 X 21 compresse. È possibile che non tutte le confezioni siano commercializzate. **6.6 Precauzioni particolari per lo smaltimento e la manipolazione.** Nessuna istruzione particolare. - **7. TITOLARE DELL'AUTORIZZAZIONE PER L'IMMISSIONE IN COMMERCIO**. Finderm Farmaceutici s.r.l Via A. De Gasperi 165/B - 95127 CATANIA . - **8. NUMERI DELLE AUTORIZZAZIONI ALL'IMMISSIONE IN COMMERCIO** - GESTODIOL 20 microgrammi/75 microgrammi compresse rivestite, - 1X21 cpr A.I.C. n.037684014/M - GESTODIOL 20 microgrammi/75 microgrammi compresse rivestite, - 3X21 cpr A.I.C. n.037684026/M - GESTODIOL 20 microgrammi/75 microgrammi compresse rivestite, - 6X21 cpr A.I.C. n.037684038/M - GESTODIOL 30 microgrammi/75 microgrammi compresse rivestite, - 1X21 cpr A.I.C. n.037684040/M - GESTODIOL 30 microgrammi/75 microgrammi compresse rivestite, - 3X21 cpr A.I.C. n.037684053/M - GESTODIOL 30 microgrammi/75 microgrammi compresse rivestite, - 6X21 cpr A.I.C. n.037684065/M - **9. DATA DI PRIMA AUTORIZZAZIONE/RINNOVO DELL'AUTORIZZAZIONE.** 2 ottobre 2007 - **10. DATA DI REVISIONE DEL TESTO.** Marzo 2010

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Embryo development using fresh and vitrified oocytes

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Introduction

In Italy zygote and embryo cryopreservation has been forbidden by law 40/2004 until the decision n. 151/2009 of the Italian Constitutional Court that affirmed the constitutional illegitimacy of several provisions of Law n. 40. Based on such conditions, in that period gamete cryopreservation became the only option for the storage of reproductive material, so it received a great effort to its development.
While cryopreservation of sperm and embryos has been an integral part of infertility treatment for some time now, on the other hand female gametes own some particular characteristics -mostly related to their anatomic structure- that make them more vulnerable. Therefore, oocyte cryopreservation has not yet become a routine procedure, and still in the 2008 the Practice Committee of the American Society for Reproductive Medicine affirmed that oocyte cryopreservation should be considered an experimental technique only to be performed under investigational protocol under the auspices of an IRB (1).

Problems and limitations of oocytes cryopreservation

Firstly, human oocytes are characterized by a low surface-to-volume ratio and a plasma membrane permeability that inhibit the passage of water and cryoprotectants, thus making it really difficult to protect oocytes from intracellular ice crystals formation (2). Secondly, mature oocytes contain the meiotic spindle, which is known to be vulnerable to the dangerous effects of temperature variation and to oocyte dehydration/rehydration (3). Moreover, early oocyte activation induced by cryoprotectants may impede subsequent development (4). Finally, both freezing and thawing expose gametes to severe stress, altering the zona pellucida, therefore impairing sperm penetration or attachment (5).
It must however be highlighted that the development of oocyte cryopreservation has not been limited to the Italian situation. Cryopreservation of oocytes has become an alternative to embryo storage for several reasons: first of all, oocyte storage has enormous potential for making oocyte donation safer (by allowing a suitable temporal window for donor screening) and for giving a hope for fertility preservation in women at risk of premature loss of ovarian function (e.g. POF, cancer patients). Nevertheless, an effective method to preserve fertility would be appreciated by all the women who, for several reasons, wish to delay pregnancy, or who prefer oocyte cryopreservation instead of embryo freezing for ethical motivations.
In human in vitro fertilization (IVF), in which the first reported pregnancy from cryopreserved oocytes was described in 1986, there are two major techniques for cryopreservation: slow freezing processes and vitrification procedures.
The most harmful effects are produced by ice crystals created during the freezing procedure. Slow freezing was the method of choice until a few years ago, but in recent years vitrification has earned increasing consideration for oocyte cryopreservation, by improving post-storage survival rates and apparently preserving more efficiently oocyte developmental ability (6). Vitrification is carried out by combining high doses of cryoprotectants with high cooling and warming rates. It has been proposed that vitrification process may be

less shocking to the meiotic spindle than slow freezing (7) and may avoid the formation of intracellular ice crystals, using cryoprotectants in high concentrations and ultra-rapid cooling and thawing procedures (8). It is important to notice that, notwithstanding the possible risks for the meiotic spindle, there seem to be no apparent increase of the incidence of chromosomal abnormalities in embryos derived from frozen eggs, and the danger is far less likely when vitrification is used (9-11). Finally, this method is cost-effective, does not require expensive freezing equipment and just a very small volume of vitrification medium is needed to store the cryopreserved cells or tissues. Despite these advantages, the main limit of vitrification is the toxic effects linked to the use of high concentrations of cryoprotectants (12). This problem has however been got over with the development of new vitrification techniques using extreme cooling rates to significantly reduce the volume of cryoprotectants needed and so the toxic and osmotic consequences.
In order to analyze with objectivity the reliability of the method, it is clearly necessary to consider the difference between the procedures used, based on type of carrier, cryoprotectant mixtures or vitrification device and temperature. Moreover, there are other variables, such as the age of the patient, and the consequent oocyte quality, or the impact of other actions like ovarian stimulation, culture conditions and embryo transfer technique.

A glance at the current literature

Over the years, the majority of the studies in this field showed some limitations, for example in relation to the use of vitrified oocytes donated by young women, which constituted a possible bias in the analysis of subsequent reproductive outcomes (12-16). The origin of the oocyte involved is, in fact, a crucial factor, with a clear advantage of oocytes produced by young donors compared to the ones coming from older women (17). Likewise, until few years ago reliable randomized, controlled trials comparing reproductive outcomes of cycles using fresh and frozen oocytes were lacking.
Since several accurate reviews in literature examine the topic of the efficiency of oocyte cryopreservation in general (18-20), we focused our attention only on the latest researches comparing the results provided by IVF cycles using fresh and frozen oocytes. Recently, some studies comparing fresh and frozen oocytes retrieved from women of comparable average age and other characteristics reported that pregnancy outcomes do not seem to be greatly affected by the vitrification procedure, as fertilization, embryo quality and further development are similar to those achieved with fresh oocytes. Especially the Cryotop system (Kitazato Supply Co., Fujinomiya, Japan), involving a minimum volume of vitrification solution, has been showed to be very effective in oocyte cryopreservation. On the other hand, in literature there are few data about results obtained with closed vitrification systems such as CryoTip (Irvine Scientific) (21). A recent study by Paffoni et al comparing an open (Cryotop) with a closed (Cryotip) device demonstrated lower outcomes for the latter (22). However, to the best of our knowledge, only one observational study was conducted comparing clinical results, so that data regarding gestational outcomes after closed vitrification are missing. The prospective observational study performed by Stoop et al used CBS High Security closed straws (Cryo Bio System) and showed comparable laboratory and clinical outcomes in an oocyte-donation programme (23). As a result, further randomized controlled trials should take into consideration closed vitrification systems as a valuable and aseptic alternative for oocytes cryopreservation. Furthermore, studies on animal models seem to confirm that cryo-survival of oocytes following vitrification procedures is reliable, but it is strongly associated with female reproductive age: the older the woman, the worst the impact on the oocytes (24). Vitrification may be responsible for inducing slight aberrations of MII configuration in old and *in vitro* aged oocytes without affecting young oocytes (25).
Even if, recently, reduced oocyte development was reported in frozen cycles in whom slow freezing was used, compared with cycles involving sibling fresh cycles (26), when vitrified oocytes are compared with fresh counterparts in an oocyte donation program, similar results are reported in terms of fertilization rates, embryo development and blastocyst formation rates (15).
Cobo et al., in 2008, published a cohort prospective randomized study where they reported their experience with vitrification using the Cryotop method. They compared the results of vitrified and fresh oocytes from the same ART cycle, and they found no significant difference in embryology data or clinical outcomes. In that study, either vitrified or fresh oocytes from the same cohort were inseminated with the same semen sample, in order to compare the sole potential of oocytes, thus eliminating any confounding factors (15). To assess also the influence of the technique on embryo development, they evaluated embryo quality in both early and blastocyst stages, that was similar between groups. We have to consider, however, that all the donors were between 18 and 35 years, so that these oocytes had a better prognosis, with respect to common infertile patients. This could represent a possible limit for the study.

Nagy et al. also examined the efficacy of oocyte vitrification using the Cryotop method. The study included 153 oocytes from 10 donors assigned to 20 recipients. When the authors compared the results of that ART cycles with those from the donors' previous fresh donation cycles, they observed very similar outcomes. Embryo development was similar in both groups and a very high percentage of blastocyst development was observed in the frozen oocytes group (27). Unfortunately, also this study examined all donors under 35 years.

In addition, Rienzi et al confirmed these results in 2010 (28), when they conducted a randomized trial comparing gestational outcomes of fresh ICSI insemination and vitrification procedure on sibling metaphase II oocytes (MII). The vitrification and warming procedures were performed with the Cryotop procedure, and, interestingly, patients were selected based on the fact that they were not older than 42. The study showed that oocyte vitrification procedure followed by ICSI was shown not to be inferior to fresh insemination procedure, with regard to fertilization and embryo developmental rates: embryo development up to day 2 was not affected by vitrification procedure, oocyte survival rate was higher than 95%, and embryo quality was similar in the studied groups.

The Cryotop method was used also by Trokoudes et al., who conducted a review of their egg-sharing program between 2007 and 2009, where the oocytes from one donor were shared by two groups of recipients, the first of them receiving vitrified donor oocytes, while the second receiving fresh donor oocytes (29). Also this study owns the limit that the age of the donors participating in this study varied between 22 and 35 years. Outcomes evaluated were fertilization rates, cleavage rates, embryo quality, and clinical outcome of both the cohort counterparts. Researchers evidenced no statistically significant difference in fertilization rates, and to estimate the influence of the procedure on embryo development, they evaluated the rates of cleavage on day 2 and good embryo development on day 3 after insemination in both vitrified and fresh oocytes, that were similar. Lastly, vitrified oocytes had similar implantation potential compared to fresh oocytes, as pregnancy rate per vitrified oocyte and implantation rate were similar to that of fresh oocytes.

In 2010, Cobo et al performed a randomized, prospective, triple-blind, single-centre, parallel-group controlled-clinical trial, in which they tested the concomitant outcome of cryopreserved and fresh oocytes. Regrettably, as well as the precedent study, the donors were all under 35 years old. The study confirmed that there was no difference between both groups, with respect to fertilization rate, embryo cleavage on Day-2 or on Day-3. Also the proportion of top-quality embryos obtained was similar in both groups. There was no significant difference also with regard to clinical outcomes, as implantation and clinical pregnancy rates per cycle and per embryo-transfer were similar for patients receiving vitrified or fresh oocytes (30).

Almodin et al., in 2010, used a novel vitrification method (Vitri-ingà), similar to Cryotop, to compare the reproductive results obtained with oocytes vitrified and thawed to that from fresh oocytes in a series of IVF-ET cycles (31). The developmental potential of embryos obtained from frozen oocytes using the Vitri-ingà process seemed not to be greatly modified by the vitrification procedure, as fertilization, embryo quality and clinical results were similar to those achieved with fresh oocytes. Only the average number of blastomeres in the fresh-oocytes-group was significantly higher.

Conclusions

In conclusion, nowadays, we are far from the assessment of a universal protocol dealing with all the knowledge accumulated until now. What's more, even though there is a growing amount of evidences sustaining the reliability and the general efficacy of oocyte vitrification, large randomized, prospective and well-controlled studies are still needed to ensure the safety of oocyte vitrification for its routine use in donation programs and for future banking, and to verify beyond all doubt its results in comparison to cycles using fresh oocytes.

Anyway, the reported studies support the hypothesis of the potential application of oocyte vitrification in a conventional IVF program. Besides, it might be useful for the treatment of infertile patients with a large spectrum of indications. Particularly, embryo development subsequent to the insemination of cryopreserved oocytes seems not to be greatly impaired by the process of cryopreservation, when it is performed with a reliable and supported technique, as vitrification.

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Ovarian cancer and assisted reproduction

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Introduction

Ovarian cancer is the leading cause of death among gynaecologic cancer and the fourth leading cause of cancer death for women (1). Lifetime risk of ovarian cancer is projected at 1.7% (2). Over 75% of cases are diagnosed at an advanced stage, with a 5-year survival rate of 30% (3).
Several hypotheses have been proposed to explain the association between ovarian cancer and reproductive history. The "incessant ovulation hypothesis", proposed by Fathalla (4), supports that the repeated cycle of damage and repair that occurs with each ovulation may lead to genetic abnormalities and thus, could contribute to the malignant transformation of the epithelial cells.
The "gonadotrophin hypothesis" (5) posits that persistent stimulation of the ovary by gonadotrophins can increase the risk of malignant changes, either by a direct carcinogenic effect or by inducing steroidogenesis. Therefore, both the "incessant ovulation" and the "increased gonadotrophin" hypotheses are consistent with the known protective effect of pregnancies and oral contraceptives (6).

Infertility: a cancer risk factor?

Several studies have investigated if infertility *per se* is an independent risk factor for ovarian cancer and this is an important issue before dealing with the potential risks of fertility drugs.

Cohort studies
Cohort studies have compared ovarian cancer rates in infertile patients with the general population, by using the standardized incidence ratio (SIR). Since cohorts of infertile women have lower parity rates than the general population and nulliparity is a strong risk factor for ovarian cancer, an increased SIR might be solely due to the confounding effect of nulliparity. Another potential limitation of most cohort studies is the small number of observed ovarian cancers and therefore, the insufficient statistical power to draw firm conclusions (Table 1).
Three studies have reported an increased risk of ovarian cancer in infertile patients (7-9). Brinton et al. found, among infertile patients, a significantly higher risk of ovarian cancer compared to the general population (SIR of 1.98 95% CI 1.4-2.6) (12); in line with these results, Rossing et al. also confirmed an increased risk (SIR 2.5 95% CI 1.3-4.5), which was somewhat higher for borderline ovarian tumors than for invasive cancer (8). The other study, which is one of the largest cohorts, describes a modestly increased ovarian cancer risk among women reporting infertility (rate ratio of 1.36 95% CI 1.07-1.75) (9).
However, this increased risk has not been consistently reported in other cohort studies (10,11). The study performed by Venn et al. included 10358 women referred for IVF treatment and revealed a non significant increase of ovarian tumors (including invasive ovarian cancer and mature cystic teratomas) in the cohort when compared to the general population. This cohort was years later expanded to 29700 women and still, the observed incidence of ovarian cancer was not greater than expected (SIR 0.99, 95% CI 0.57-1.70) (12).
Jensen's study was the first to determine parity-specific and parity-adjusted SIRs for ovarian cancer in a cohort of infertile women (13). The results of this cohort, including 54362 infertile women, revealed

TABLE 1 - COHORT STUDIES: INFERTILITY AND OVARIAN CANCER RISK.

	Size	Mean follow-up (yrs)	Patients	No. of cases	Risk (95% CI)
Brinton et al. (7)	12193	18.8	Evaluated for infertility	45	SIR 1.98 (1.4-2.6)
Rossing et al. (8)	3837	6.9	Evaluated for infertility	11	SIR 2.5 (1.3-4.5)
Tworoger et al. (9)	107900	28	Infertile, not male factor	612	RR 1.36 (1.07-1.75)
Venn et al. (10)	29700	8.5	Evaluated for infertility	13	SIR 0.99 (0.57-1.70)
Modan et al. (11)	2496	21.4	Diagnosed with subfertility	12	SIR 1.6 (0.8-2.9)
Jensen et al. (13)	54362	13	Evaluated for infertility	155	SIR 1.46 (1.24-1.71)

SIR: standardized incidence ratios
CI: confidence interval
RR: relative risk

that infertile women have increased risk of ovarian cancer, even when results were adjusted for parity status.

Case-control studies

Two large pooled analyses have suggested that infertile women with long periods of infertility might be at increased risk for ovarian cancer (14,15) (Table 2). Whittemore et al. reported that a history of more than 15 years of unprotected intercourse was associated with an increased risk of ovarian cancer, both among gravid and nulligravid women, when compared to those with less than 2 years of unprotected intercourse. The other pooled analysis found that, among nulligravid women, attempts for more than 5 years to become pregnant compared with attempts for less than 1 year increased the risk of ovarian cancer 2.67-fold (95% CI 1.91, 3.74) (15).

In agreement with these results, a more recent case – control study (16), which included 378 cases and 1637 controls, found no association of cancer risk with a history of infertility or specific types of infertility among parous women, however, this risk was increased among nulliparous women (odds ratio = 1.6, 95% CI 1.0-2.6)

It seems therefore, that infertility may increase the risk of ovarian cancer in those patients who remain nulligravid despite long periods of unprotected intercourse.

Infertility origin and ovarian cancer risk

The association between specific causes of infertility and ovarian cancer has also been addressed by several studies. Ovulatory disorders, endometriosis and unexplained infertility are the most common diagnosis associated to ovarian cancer (17); however these associations have been inconsistently observed.

Endometriosis seems to be the subtype of infertility where studies agree most. Brinton et al., in their cohort of 12193 infertile women, observed no increased ovarian cancer risk associated with ovulatory, tubal or male infertility; however, an increased risk was observed with endometriosis (RR 2.27) (18). Melin and co-workers (19) have further investigated the possible association between endometriosis and ovarian cancer after adjusting for parity. They included 63630 women who had been discharged from hospital with a diagnosis coded for endometriosis with a long follow-up period. An increased risk of ovarian cancer was observed (SIR 1.37, 95% CI 1.14-1.62) when compared to the general population. Some limitations to Melin's study are that information regarding prior fertility drug use is lacking and that only moderate to severe endometriosis cases were included.

TABLE 2 - CASE-CONTROL STUDIES: INFERTILITY AND OVARIAN CANCER RISK.

	No. of cases	No. of controls	Comparison	OR (95% CI)
Whittemore et al. (14)	2197	8893	12 C-C studies	Nulligravid: 1.4 (0.86-0.23) Gravid: 0.87 (0.67-1.1)
Ness et al. (15)	5207	7705	8 C-C studies	Nulligravid: 1.19 (0.91-1.55) Gravid: 1.16 (1.02-1.31)
Rossing et al. (16)	378	1637		Nulliparous: 1.6 (1.0-2.6)

CI: confidence interval
OR: odds ratio

Use of fertility drugs and ovarian cancer risk

Given the fact that fertility drugs raise temporarily the levels of gonadal hormones and gonadotrophins and that hormonal and reproductive factors are known to be involved in the aetiology of ovarian cancer, a stimulating effect of fertility drugs in the risk of this cancer is theoretically possible and compatible with both the "incessant ovulation hypothesis" and the "gonadotrophin hypothesis".

Cohort studies

Rossing et al. examined a cohort of 3837 infertile women and found 11 ovarian tumors (invasive or borderline), with a relative risk (RR) of 2.5 (95% CI 1.3-4.5). The risk was higher for borderline tumors and was more pronounced with prolonged use of CC (RR 11.1, 95% CI 1.5-82.3 after 12 or more cycles, among women with and without ovulatory problems and independent of parity status). However, these results are difficult to interpret if we take into consideration the small number of cases, with almost half of them being borderline tumors, and the limited information on confounding factors (8) (Table 3).

Other cohort studies have not found an increased risk of ovarian cancer among infertile patients who used fertility drugs compared to the unexposed group (11-20), however, only one of this studies controlled for parity status.

One of the largest cohort studies evaluating the association between fertility treatment and ovarian cancer included 10358 infertile women referred for IVF treatment (10). Ovarian cancer incidence was not greater than expected in the exposed group.

A more recent cohort study including a large cohort of infertile women (12193 women) with a long follow-up and information available regarding specific causes of infertility, parity, oral contraceptive use and other confounders, reports that women who had been exposed to fertility drugs (both CC and gonadotrophins) were not at an unusual risk of developing ovarian cancer compared to women who had never been exposed to either drug; concluding that even though results were reassuring, there is a need for continued monitoring of long term effects (7).

Another cohort study including only parous women did not find an association between use of ovulation inducing agents and ovarian cancer, although numbers were small (only one exposed patient among 43 cancer cases) and data regarding type of infertility and type, doses and duration of treatment were absent (21).

Jensen and colleagues, in their cohort of 54362 women, found no convincing association between use of fertility drugs (CC, hCG, gonadotropins, GnRH analogues) and the overall risk of ovarian cancer. Risk did not differ according to any use of fertility drug, number of cycles, length of follow-up since first drug use or parity. They did find an increased risk of ovarian cancer in infertile women, even after adjusting for parity, suggesting therefore that other factors related to infertility rather than the use of fertility drugs are involved in this risk increase. When they analysed differences according to histological subtype, a 67% increased risk for serous ovarian cancer was found after use of CC, primarily among women followed for 15 years or more (22).

TABLE 3 - COHORT STUDIES: FERTILITY DRUGS AND OVARIAN CANCER RISK.

	Size	Mean follow-up (yrs)	No. of cases	Comparisons	Risk (95% CI)
Rossing et al. (8)	3837	6.9	11	Treatment vs general population CC use vs no use CC use vs no use	SIR 2.5 (1.3-4.5) RR 2.3 (0.5-11.4) RR 1.0 (0.2-4.3)
Modan et al. (11)	2496	21.4	12	Treatment vs general population	SIR 1.7 (0.6-3.8)
Doyle et al. (20)	5556	8.5	6	Treated vs untreated	RR 0.59 (0.12-3.00)
Venn et al. (12)	29700	7 for exposed group	13	Treated (IVF) vs general population	SIR 0.88 (0.42-1.84)
Brinton et al. (7)	12193	18.8	45	CC use Gonadotropin use	RR* 0.82 (0.4-1.5) RR* 1.09 (0.4-2.8)
Calderon Margalit et al. (21)	15030		43	Ovulation inducing agents	HR 0.61 (0.08-4.42)
Jensen et al. (22)	54362	16	156	Ovulation inducing agents	RR* 1.03 (0.73-1.47)
Sanner et al. (23)	2768	33	17 (invasive cancers)	Gonadotropins vs no use CC vs no use	RR 5.28 (1.70-16.47) RR 1.57 (0.32-7.62)

CI: confidence interval
HR: hazard ratio
SIR: standardized incidence ratio
RR*: rate ratio
RR: relative risk

In contrast with the previous results, a recently published cohort study with a mean length of follow-up of 33 years, found an increased risk of invasive ovarian epithelial cancer in association with gonadotrophin treatment in women with non-ovulatory disorders when compared to unexposed women (RR 5.28, 95% CI 1.70 – 16.47). However, these findings must be interpreted with caution as the small number of cancer cases makes statistical analysis imprecise with wide confidence intervals (23).

Case-control studies

The first large case – control study reporting on a possible association between fertility drugs and ovarian cancer was the meta-analysis performed by Whittemore and colleagues (14). They found a 3 fold increase in the risk of developing an ovarian cancer with the use of fertility drugs, when compared to women without a history of infertility (OR 2.8, 95% CI 1.3-6.1) (Table 4).

However, the article provides no information about reasons for infertility, the type of fertility drugs used, nor the dosage or duration of treatment.

Some of these limitations are also met in Shushan's study (24), where the use of fertility drugs, particularly hMG, was associated with an increased risk of ovarian cancer (OR 3.95, 95% CI 1.33-12.2), being the association stronger for borderline ovarian tumors (OR 9.38, 95% CI 1.66-52.08).

In contrast with these results, Moosgard et al. did not find an increase of ovarian cancer risk among parous and nulliparous women treated with fertility drugs when compared to non treated (25). This study was one of the eight case – control studies included in a large pooled analysis (15), which concluded that there was no association between fertility drug use and the overall risk of ovarian cancer.

Parazzini's study showed reassuring evidence of the absence of a strong association between the use of fertility drugs and subsequent risk of ovarian cancer (26).

In another case – control study, Rossing et al. did not find an association between fertility drugs use and the risk of ovarian cancer among either parous or nulliparous women (16), which is in disagreement with the findings of a previous cohort study conducted by the same authors (8).

A meta-analysis evaluating the risk of assisted reproductive technology (ART) and ovarian cancer which analysed separately cohort and case – control studies (7 case – control and 3 cohort studies), found in the cohort data that risk was not increased in treated patients, even more, they found a trend towards a decreased risk, which could be explained by the fact that ART may result in pregnancy and parity is a protective factor against ovarian cancer. Data from case – control studies included in the meta-analysis showed no effect of fertility treatment, but confounders were not taken into account. A strength of this meta-analysis is that they compared the incidence of ovarian cancer in treated infertile vs. untreated infertile patients (27).

Conclusion

In conclusion, female infertility may be associated with an increase in ovarian cancer risk in those patients who remain nulligravid despite long periods of unprotected intercourse.

Regarding the potential risk of fertility drugs, findings seem to be reassuring, as most of the studies have shown no overall increase in ovarian cancer risk; however, further research is warranted as some studies have reported an increased risk of ovarian cancer with greater exposures or extended follow-up.

TABLE 4 - CASE-CONTROL STUDIES: FERTILITY DRUGS AND OVARIAN CANCER RISK.

	No. of cases	No. of controls	Comparison	OR (95% CI)
Whittemore et al. (14)	526	966	Fertility drugs vs. no infertility	OR 2.8 (1.3-6.1) Nulligravid: OR 27 (2.3-315.6) Gravid: OR 1.4 (0.5-3.6)
Shushan et al. (24)	164	408	Any drug vs. no use hMG vs. no use	OR 1.31 (0.63-2.74) Borderline: OR 9.38 (1.66-52.08) Invasive: OR 3.19 (1.33-12.2)
Moosgard et al. (25)	684	1721	Fertility drugs vs. no use	Nulliparous: OR 0.83 (0.35-2.01) Parous: OR 0.56 (0.24-1.29)
Ness et al. (15)	1060	1337	Fertility drugs vs. no use	OR 0.97 (0.76-1.25)
Parazzini et al. (26)	971	2758	Fertility drugs vs. no use	OR 1.1 (0.4-3.3)
Rossing et al. (16)	108 nullip. 270 parous	343 nullip. 1291 parous	Fertility drugs vs. no use	Nulliparous: OR 1.0 (0.4-3.0) Parous: OR 0.8 (0.4-1.6)

CI: confidence interval
OR: odds ratio

Specific attention should be focused on effects among women with long-term subfertility, above all among those who remain nulligravid.
Well-conducted studies with large sample sizes, long follow-up periods and data on confounding factors, the type of infertility and the type, dosages and duration of treatment, as well as on histology of tumors, are needed (28-30).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Effects of life-style and metabolic factors on post-menopausal endometrium

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Introduction

In Western countries women the endometrium is frequently exposed, even after menopause, to the action of endogenous estrogens. Such a stimulation, strongly linked to women's metabolic pattern, increases the risk of pathologic conditions such as endometrial hyperplasia and type I (endometrioid) endometrial adenocarcinoma. Obesity, type II diabetes and the metabolic syndrome, for instance, promote the endometrial stimulation. As a consequence, the dietary and lifestyle changes that reduce obesity and metabolic disequilibrium may act as important preventive factors for the endometrium disorders.

The postmenopausal endometrium

In asymptomatic postmenopausal women undergoing hysterectomy for a prolapsed uterus, the endometrium frequently shows some features of activity (1,2); even when atrophic, 50% shows various degrees of proliferative and angiogenic activity, either diffuse or focal (2). Similar activities has been also shown by the non-neoplastic atrophic endometrium adjacent to type I estrogen-dependent adenocarcinoma (2,3). Actually, cancer is more frequently associated with patterns suggesting a more definite endometrial stimulation. Compared with women who present "disordered proliferative endometrium" (considered equivocal hyperplasia), the women with "simple hyperplasia" have a non-significant increased risk of cancer (Relative Risk = 2.0, 95% CI, 0.9-4.5); the women with "complex hyperplasia" have a significant increased risk of cancer (2.8, 1.0-7.9) and the women affected by the precancerous "atypical hyperplasia" have a Relative Risk = 14.2 (5.3-38.0) (4). Over 80% of cases of endometrial cancer occurs after menopause; among these, 75% are the type I tumours, while 25% are non-endometrioid estrogen-independent cancers (3,5). Therefore, in post-menopausal women, the majority of endometrial pathology is a consequence of a continuous low level estrogenic stimulation and epidemiologic data suggest that such a stimulation is associated with metabolic and life-style factors.

Risk factors connected with life-style and/or metabolic alterations

The association between endometrial cancer, metabolic alterations and lifestyle factors is well recognized (6). The incidence of endometrial cancer is higher in Western, industrialized countries than in rural Asia or Africa. Observing the changes in incidence rates over time, after industrial progress or migration from low to high risk areas, it is clear that endometrial cancer is strongly related to westernised diets, obesity and sedentary lifestyles (7).

Obesity

Postmenopausal obese women have a 3-4 fold increased risk of complex hyperplasia and hyperplasia with atypia (8-10). Regarding endometrial cancer, evidence indicates that 40-50% of endometrial cancers may be due to too much body fat, suggesting that energy balance has a critical role in the aetiology of this disease (11,12). Different studies showed that obesity is associated with a 2-5-fold increased risk in both pre and post-menopausal women (5,7).

Diabetes
A trend to increased risk of endometrial hyperplasia with atypia (4.0; 0.8-19.1) was observed in diabetic women aged 52 years or more (8).
Epidemiological studies consistently showed that women affected by noninsulin-dependent diabetes have an increased risk of endometrial cancer both before and after menopause (5,7). This increased risk is independent from obesity, as it persists after controlling for body mass index (BMI) (13).

Sedentary behaviour
The World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) Report, reviewed the totality of epidemiological evidence on physical activity and endometrial cancer relation, concluding that physical activity probably reduces endometrial cancer risk (14). However, five large prospective cohort studies and a meta-analysis (15) have since published findings on the relationship between physical activity and endometrial cancer (16-20). The totality of evidence now convincingly indicates that physical activity prevents or reduces risk of endometrial cancer (active women having an approximately 30% lower risk than inactive women) and this effect is independent of adiposity. Consistently, sedentary behaviour is emerging as a risk factor in a few studies that evaluated the risk associated with sitting for more than 5 hours/day (15-21).

Mechanisms of action

Endometrial hyperplasia (and the subsequent cancer) arise when estrogen, unopposed by progesterone, stimulates endometrial cell growth by binding to estrogen receptor and by increasing the local production of Insulin-like Growth Factor-I (IGF-I) (7).
Progesterone contrasts the estrogen effects and prompts the shedding of endometrial tissue by reducing the number of estrogen receptors, by increasing the rate of conversion of estradiol (E2) to the less potent estrone (E1) and by favouring the expression of IGF Binding Protein-1 (IGFBP-1) which inhibits IGF-I action in endometrial tissue (7).
Unopposed high estrogen levels are a risk factor for endometrial hyperplasia, as shown by the results of the randomized Post-menopausal Estrogen/ Progestin Interventions (PEPI) Trial, where women assigned to receive estrogen-only therapy were more likely to develop simple, complex or atypical hyperplasia than women treated with placebo (27.7% vs 0.8%, 22.7% vs 0.8% and 11.8% vs 0%, respectively) (22).
However, endogenous estrogens in post-menopausal women, even if at the top of the range as in obese women, do not completely explain the association between life-style and metabolic alterations and hyperplastic or neoplastic endometrial pathology. Other factors in addition to estrogens, such as high serum levels of insulin, may promote the proliferation of endometrial tissue.

Endogenous estrogens and androgens in postmenopausal women
After menopause, androstenedione and testosterone are converted into estrone and estradiol through the action of aromatases in the peripheral tissues, especially in the adipose tissue (5,23). After menopause, androgens mainly derive from the adrenal cortex.
In detail, the reticolar area of the adrenal cortex produces dehydroepiandrosterone (DHEA), which, especially as dehydroepiandrosterone sulphate (DHEAS), represents the major circulating C19 steroid (24). DHEAS and DHEA are converted in the peripheral tissues, especially in the adipose tissue, into the delta4-steroids androstenedione and testosterone (25-27). Furthermore, the ovary contributes to the production of DHEA (24) and testosterone (20-40% of the total synthesis) (28-30).
Obesity does not influence levels of DHEA/DHEAS but is the major determinant of levels of delta4-steroids. Epidemiological data showed that obese women have higher serum levels of androstenedione, testosterone, and free testosterone (29). “In vitro” and “in vivo” data suggest that androgens may have no effect or inhibitory effect on endometrial cell proliferation (31,32); however, through the conversion into estrogens androgens may represent a risk.
Elevated circulating androgens have been associated with endometrial hyperplasia (7). Several studies found that women with type I endometrial cancer have higher levels of both estrogens and androgens (33). Prospective studies showed that endometrial cancer risk in postmenopausal women is positively associated with levels of estrogens and androgens, and inversely associated with SHBG, even after adjustment for BMI (34,35).

Insulin
Type II diabetes (non-insulin dependent) is characterized by hyperinsulinemia due to insulin resistance and is associated with an increased risk and mortality of endometrial cancer (7,36). Epidemiological data on postmenopausal women suggested also an increased risk of endometrial cancer in non diabetic women with hyperinsulinemia (7). Insulin may have important direct effects, working as a growth factor, similar to those of IGF-I (7,37). Elevated insulin could also increase IGF-I activity in endometrial tissue by suppressing gene expression of endometrial IGFBP-I (7)

Moreover, insulin reduces the liver production of SHBG (29,38) and hyperinsulinemia due to insulin resistance is associated to high serum levels of testosterone in most studies (39). Insulin stimulates the ovary and adrenal cortex production of androgens (especially androstenedione and testosterone) through the 17α-hydroxylase and 17,20-lyase activities (40).

Other mechanisms

It has been hypothesized that *inflammatory mechanisms* partially mediate the relationship between obesity and endometrial cancer risk. In a large prospective study levels C-reactive protein (CRP) were positively associated with the risk of endometrial cancer (41). Interestingly in this study, the excess risk due to obesity was reduced by 48%, 67% and 77% when either E1, CRP or insulin, respectively, was included in the model; the risk became null in the model that simultaneously adjusted for all these three factors.

Epidemiologic studies suggested also that low levels of *adiponectin* contribute to mediate the relationship between obesity and endometrial cancer (42,43). Adiponectin opposes insulin resistance and inflammatory factors. Normal endometrium, even if atrophic, and endometrial cancer tissue, express adiponectin receptors (43); adiponectin may also affect cancer development directly, at a local tissue level, through the activation of the adenosine 5'-monophosphate-activated protein kinase (AMPK) (43).

Prevention of endometrial pathology

A diet aimed at lowering calories and insulin levels (low fat, refined carbohydrates and animal products, high whole grain cereals, legumes and vegetables) and physical activity reduce body weight and the metabolic syndrome, improves insulin sensitivity, and decreases the bioavailability of sex hormones and growth factors. A "lifestyle" intervention may be probably useful for prevention of endometrial pathology.

Intentional weight loss causes an increase in SHBG levels and a decrease in levels of insulin, CRP, androgens and estrogens (12,44), with a one-third reduction in free E2 to be expected from a 10% weight loss (12). Observational cohort studies and randomized controlled trials of both dietary interventions and bariatric surgery indicated fairly immediate reductions in cancer incidence following intentional weight loss (12).

Physical activity reduces insulin level and insulin-resistance independently of its influence on BMI (45). Higher levels of sport activity are associated with lower levels of estrogens and androgens (46,47); however, some data suggested a non-linear relationship between recreational physical activity and hormone levels in postmenopausal women (48). Anyway, findings of five large prospective cohort studies consistently suggested that physical activity protects against endometrial cancer, and this effect is independent of adiposity (15).

Based on these studies, one hour daily of moderate-intensity activity (e.g. walking) significantly reduces the risk of endometrial cancer (15,21); 74% of European population is insufficiently active and 34% is sedentary (21). Approximately 30000 cases of endometrial cancer could have been prevented in 2008 in Europe (2700 in Italy alone) if the population had maintained sufficient levels of physical activity (21).

In addition to life-style modifications, the antidiabetic Metformin, a "caloric restriction mimetic" with a possible anticancer activity (49), may be proposed as preventive agent in women with metabolic syndrome, pre-diabetes and/or obesity. Metformin inhibits liver neoglucogenesis by activating AMPK, and reduces insulin level (49). Furthermore, data from our randomized trial showed a 23% reduction in testosterone levels in postmenopausal breast cancer patients treated with 1500 mg/day of Metformin (50). Metformin could also have direct anticancer effect, mainly by activating AMPK, thus mimicking the effect of calorie-energy restriction, which reduces all energy consuming processes in the cells including cell proliferation (49). Preclinical data showed that Metformin is a potent inhibitor of endometrial cancer cell proliferation, an effect partially mediated through AMPK activation (51).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Management of Turner's syndrome in adult life

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Introduction

Turner's syndrome (TS) is the most common chromosomal abnormality in females, and affects 1 in 2500 live female births. This condition is more common *in utero*, affecting 1-2% of all conspectuses. Only 1% of fetuses do not end up in miscarriage (1). TS is the result of the absence or the abnormality of the second sexual chromosome, in at least one cellular line. The chromosome might be completely lost (45X0), may have undergone duplication of the long arm with the loss of the short one (isochromosome Xq), may transform intro a ring formation (rX), or suffer a deletion that includes the tip of the short arm. Monosomy 45X0 is the most frequent form in peripheral blood lymphocytes (40-60%), but other karyotypes present mosaic patterns like 45X0/46XX, 45X/46XY or 45X/46Xq- (Table 1, Fig. 1).

TS is associated with a wide array of potential abnormalities, most thought to be caused by haploinsufficiency of genes that are normally expressed by both X-chromosomes (2). The cardinal features of TS are short stature and ovarian failure with insufficient sex steroids. These dysfunctions cause delayed puberty and primary amenorrhoea in most cases. Most medical attention has therefore been focused on early diagnosis, looking for signs for prenatal diagnosis, or performing paediatric guidelines for treatment with growth hormone (GH) and pubertal management (3). Nowadays, it has become evident that patients with TS are susceptible to some disorders whose beginning or evolution occurs in adult life, such as osteoporosis, hypothyroidism, diabetes, dyslipemia or non congenital cardiac or nepho-urological changes. Morbidity and mortality are increased, and life expectancy is reduced mainly by cardiovascular diseases (1,3-7).

TABLE 1 - KARYOTYPE DETERMINED IN BLOOD SAMPLES OF PATIENTS IN THE GYNAECOLOGICAL ENDOCRINOLOGY UNIT OF HOSPITAL CLÍNIC OF BARCELONA.

Karyotype		**N Patients (%)**
Pure monosomies	45 X0	10 (32%)
Deletions	46XdelX	2 (6%)
Translocations	46,XX t(X;7)(q26:p13)	1 (3%)
Isochromosome	46 X i(X)(q10)	2 (6%)
Mosaicisms		
Pure / XX	Mos 45X0/46XX	3 (10%)
Ring chromosome / XX	Mos 45XrX/46XX	3 (10%)
Pure / isochromosome	Mos 45X0/46XiXq	9 (29%)
Pure / XY	Mos 45X0/46XY	1 (3%)

Special care during adulthood is necessary, with co-ordination among different specialties, in order to develop guidelines for the correct control of sensorineural and endocrine disorders, to seek associated malformations, and for reproductive counselling or sexual health. Gynaecologists should take primary responsibility for the management of these patients to maintain and control hormone replacement, referring them to other specialties if required. In this systematic review, the complications of patients with Turner's syndrome in adult life are described.

Clinical features

Short stature and gonadal dysgenesis are the main clinical stigmata, accompanied or not by other dysmorphology secondary to lymphoedema (5,6). An association between karyotype and phenotype exists, but it is not predictable. For instance, external dysmorphology and nephrologic or cardiac malformations are common in pure monosomy (1,8), while 40% of patients

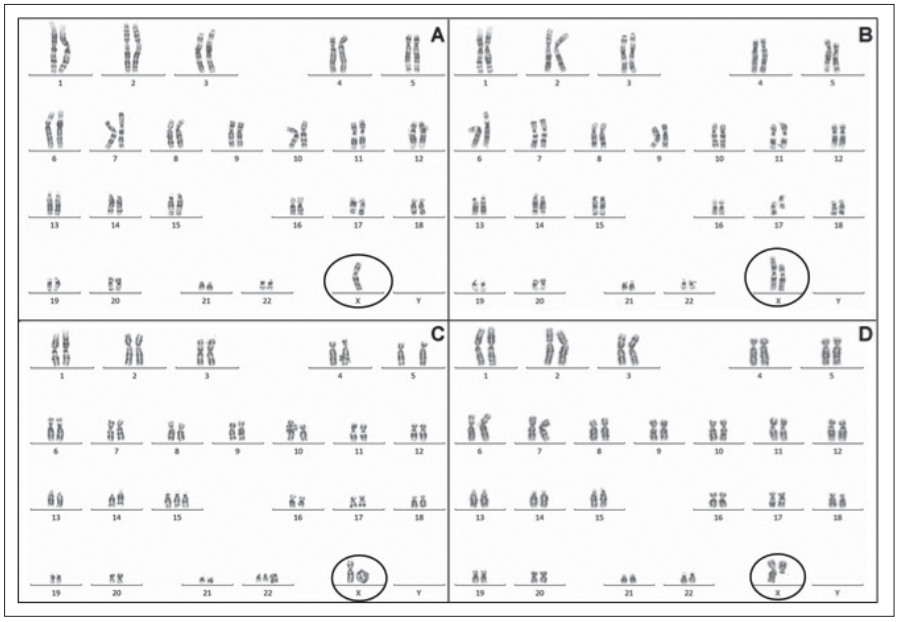

Fig. 1 - Examples of Turner's Syndrome (X-chromosome abnormalities); A) monosomy 45X0; B) isochromosome X; C) X-ring chromosome; D) X-delection.

with mosaic patterns present spontaneous menarche with fewer external features. In addition, isochromosome is associated with sensorineural and immunological disorders, without congenital malformations.

Short stature is almost an invariable finding in women with TS, with a mean final adult height between 143 and 147 cm (Fig. 2). The cause is thought to be due to a primary bone defect, associated with a partial growth hormone (GH) insensitivity. Therefore, patients receiving treatment during childhood with GH therapy for a mean of 5.7 years were on average 7.2 cm taller than the control group (9). It is recommended to begin GH treatment 4 years before oestrogen replacement (1), and the addition of oxandrolone associated with GH seems to increase height gain and slow breast development without affecting the body mass index (10). Two genes have been identified as strong candidates for short stature, SHOX and PHOG, both located in the distal part of the short arm of the X- and Y-chromosomes (Xp11-22, Yp11). Mutations in these genes could also explain some skeletal abnormalities in TS, such as Madelung deformity of the wrist, cubitus valgus or short fourth metacarpal. Haploinsufficiency of SHOX expression could explain other features such as chronic otitis media, prominent ears, and problems learning how to suck, blow, eat or articulate (2,11).

Lymphoedema is caused by an absence or hypoplasia of lymphatic vessels and is generally identified at birth, improving overtime. Other external physical disorders linked to TS are epicanthus, deformity in the pinna, micrognathia, cleft palate, short neck, pterygium colli, short limbs, low-set ears, cubitus valgus and genu valgum (4).

Ovarian dysgenesis

Although the gonads in TS differentiate normally until the third month of gestation, an accelerated degeneration of oocytes occurs after this period, with an increase in ovarian stromal fibrosis. Consequently, ovarian failure takes place within the first few months or years of life. Despite the fact that primary amenorrhoea is usual in TS, the incidence of spontaneous puberty is 8% in patients with the 45X0 karyotype, while it is found to be as high as 40% in women with mosaicism (5). Concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are high as early as 5 days of age in infants with TS; although these levels decline afterwards, FSH and LH levels remain higher than in girls with a normal karyotype (6,12).

In up to 6% of TS patients the karyotype includes the Y-chromosome, which may lead to the development of

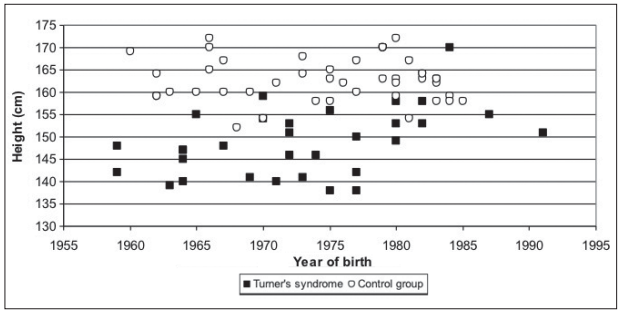

Fig. 2 - Distribution of the final height according to the year of birth of Turner's syndrome patients followed in Hospital Clinic (black squares) and age-matched controls (white circles).

gonadoblastoma, a malignant neoplasm composed of stromal and germ cells. Hence, early prophylactic excision of the gonads is recommended in TS women, considering that the risk increases with age (from 2% at age 10, to 27.5% at 30 years) (5).

Hormone replacement therapy

After the induction of puberty with oestrogens, most females with TS require long-term oestrogen replacement therapy with the aim of preventing osteoporosis, reducing risk factors for atherosclerosis and improving aspects of cognitive function (5,13).

Replacement treatment is recommended with natural oestrogen preparation and to be administered orally or with transdermal patches, taking into account the patient's preference or to avoid first-pass hepatic metabolism. Ethinyl-estradiol presented in contraceptive pills has been associated with adverse effects on liver enzymes, lipid metabolism and blood pressure, both in women with normal and Turner karyotype. In addition, some TS females are symptomatic during the pill-free week because of the complete lack of oestrogens.

Progestagen should be given for a minimum of ten days per month in order to prevent endometrial carcinoma, even though a continuous regimen would avoid menstrual bleeding.

Oestrogen deficiency causes bone loss, endothelial dysfunction, decreased insulin production, an abnormal lipid pattern, increased central adiposity and early atherosclerosis. In oestrogen deficient females with TS, replacement therapy improves liver enzyme abnormalities and some cognitive deficits (reaction time, non verbal processing speed, short term memory) (14). Therefore, the use of oestrogen replacement up to physiological doses should be maintained until the expected age of menopause. Nonetheless, it is important to emphasize that neither the risk of breast cancer nor that of ovarian or endometrial cancer is higher in these patients than in the general population (6).

Fertility and pregnancy

Concerning options for fertility, spontaneous pregnancies occur in less than 5% of TS patients, with the risk of the development of congenital malformations or chromosomopathies. Oocyte donation and *in vitro* fertilization should be recommended because most of women with TS are infertile. Nevertheless, the risk of first trimestre miscarriage is higher, probably due to uterine hypoplasia and some uterine ischaemia during pregnancy (12,15,16). Caesarean rates are higher due to cephalopelvic disproportion resulting from their body habitus.

TS is associated with many cardiovascular disorders and as such, cardiac assessment, including echocardiography and strict blood pressure monitoring should be recommended before the application of assisted reproductive techniques.

Finally, prenatal diagnosis of TS allows the prediction of gonadal insufficiency in women demonstrating early evidence of ovarian function; hence, new techniques of ovarian tissue cryopreservation with the aim of replantation may be suitable for a few selected females with TS.

Osteoporosis

Short size and osteoporosis could be due to a primary defect in bone formation. Although this molecular defect remains to be identified, some genes situated in X-chromosome are associated with connective tissue changes (17).

A reduction in peak bone mass by 25% has been described in women with TS. Nonetheless, bone density measurements depend on height, and short size and GH treatments in TS may be confounding factors. However, the incidence of fracture in girls and adult women with TS is 3-fold higher than in normal controls (18).

GH treatment for at least one year, together with oestrogen replacement started before 12 years of age, improve bone mineral density. Indeed, girls with TS and spontaneous menarche have been found to achieve normal bone mass (19).

Cardiovascular diseases

Along with all the alterations described above, cardiovascular complications are the main cause of increased mortality in TS, in which life expectancy may be reduced by up to 13 years. Dilatation of the root of the aorta, hypertension, and bicuspid aortic valve have been reported as major cardiovascular complications in TS (20). In addition, mortality due to ischaemic heart disease is increased up to 7-fold in women with TS, although the precise mechanisms of increased cardiovascular risk in TS are unclear (20).

Despite cardiovascular complications in TS being mainly associated with hypogonadism, and a consequent decrease in oestrogen production (21), differences in X-chromosome gene expression may also contribute to these complications. Several genes implicated in the control of cardiovascular function have been described in the X-chromosome, including the angiotensin type 2 receptor and several kinases and transcription factors (22).

Bicuspid aortic valve is the most common congenital malformation (16%) and, although it is usually an iso-

lated abnormality, it may occur together with other anomalies such as aortic coarctation. This combination may result in progressive valvular dysfunction due to calcification in the aortic valve and may cause aortic stenosis or regurgitation in adulthood. Coarctation of the aorta affects 10% of women with TS causing hypertension and seems to be more associated with severe lymphoedema, perhaps due to abnormal lymphatic flow by compression of the ascending aorta (2,23).

Other abnormalities, such as partial anomalous venous drainage and mitral valve prolapse are more common among TS women, and left-side cardiac anomalies are associated with endocarditis, with prophylactic antibiotics being essential before surgical procedures.

However, the most serious risk for females with TS is aortic dissection, which may occur at any age causing even sudden death. Accordingly, echocardiography should be included in the assessment of TS patients and should be indicated periodically. Electrocardiogram should be carried out along with the imaging studies because conduction or repolarization defects have been reported attributed to neuroautonomic dysfunction (2).

Hypertension, a bicuspid aortic valve and dilated aortic root (with an age-related increase of the root diameter greater than the normal population) are risk factors for dissection, making antihypertensive treatment in women with two of these three disorders advisable (24).

Metabolic abnormalities

Patients with TS have a higher prevalence of other subrogate cardiovascular risk factors such as dyslipidemia and diabetes mellitus due to insulin resistance (5). Type 2 diabetes mellitus is 2-4-fold more common in women with TS, and an early metabolic defect in glucose uptake has been described. Insulin resistance and secondary hyperinsulinemia do not seem to be dependent on the body mass index, as in the polycystic ovarian syndrome, but obesity and an elevated waist-to-hip ratio found in TS worsen this defect. Moreover, hypertriglyceridemia may be a consequence of hyperinsulinemia and obesity.

Hypercholesterolemia has been described at the expense of an increase of low density lipoproteins followed by a decrease of high density lipoproteins. GH therapy aggravates this disorder, but the effect is reversible after discontinuation of the treatment (25).

Liver enzymes are often raised in females with TS, but this seems to be a transient and benign dysfunction (Table 2). However, these patients have a 5-fold increase in the risk of cirrhosis (1). Hence, liver function should be checked periodically.

TABLE 2 - CARBOHYDRATE , LIPID AND HEPATIC PROFILE PATIENTS (N=31) IN THE GYNAECOLOGICAL ENDOCRINOLOGY UNIT OF HOSPITAL CLINIC OF BARCELONA.

	Average ± SD	Number of patients above normality limit
Carbohydrate profile		
Glucose	77,4 ± 12,3	1[a]
Insulin	9,0 ± 5,6	3
Lipid profile		
Cholesterol	196,2 ± 36,5	2[b]
HDL-cholesterol	63,7 ± 14,4	0
LDL-cholesterol	116,2 ± 30,8	0
Triglycerides	77,5 ± 29,2	0
Hepatic profile		
ASAT[c]	32,8 ± 22,4	6
ALAT[d]	39,8 ± 32,6	9
GGT[e]	96,9 ± 138,7	15
Alkaline phosphatase	235,6 ± 130,8	10
LDH[f]	423,5 ± 61,9	8

[a]Patient diagnosed of Diabetes Mellitus type II. Receiving treatment with metformin. [b]One of the cases belonged to the group of 9 patients receiving statins for previous hypercholesterolemia. The other one has never been treated for hypercholesterolemia. [c]ASAT: alanine transaminase, [d]ALAT: aspartate transaminase, [e]GGT: gamma-glutamil transferase, [f]LDH: lactate dehydrogenase.

In conclusion, close control of blood pressure, as well as a yearly determination of the lipid, carbohydrate and hepatic profiles should be included in the correct assessment of TS women in adulthood.

Immunological disorders

Hypothyroidism affects up to 70% of TS patients, especially due to autoimmune causes, and a mild and transient TSH elevation without thyroid autoantibodies has been observed. Thyroid function should be checked annually in order to diagnose the development of hypothyroidism early.

Indeed, autoantibodies and consequently, autoimmune diseases, such as coeliac disease, inflammatory bowel disease, *vitiligo* and *alopecia areata*, adrenal insufficiency, juvenile idiopathic arthritis or type 1 diabetes mellitus, are increased in these patients (26,27,28). A region in the X-chromosome where the foxp3 gene, which defines IPEX syndrome may be found, has been linked to these autoimmune disorders. FoxP3 expression correlates with CD127low leucocytes (leucocytes with low CD127 expression) (29), and it has been suggested that the same alteration may justify an increase in the prevalence of autoimmunity in TS, especially among isochromosome karyotypes (29).

It has also been suggested that other immune disorders may be present in TS patients; for instance a low CD4/CD8 ratio or a low concentration of im-

munoglobulins. Despite these alterations, an increase in infections has not been described, with the exception of otitis media (29,30).

Sensorineural disorders

Young and middle-aged women with TS have a progressive type of hearing impairment, deteriorating rapidly in adult age. The hearing decline seems to consist of two patterns: a mid-frequency dip, and a high-frequency loss resembling age-related hearing impairment (31). The conductive hearing loss seems to be a consequence of several episodes of otitis media during infancy. The main cause for the infection is the deformity in the pinna, more pronounced in patients with a total delection of the short arm of the X chromosome, as monosomy 45X0 or isochromosome (32). Therefore, the conductive loss may have a genetic origin. However, the pathophysiology of sensorineural lesions is not yet fully understood. Some studies indicate that cochlear dysfunction is the cause of the sensorineural impairment, and it is possibly influenced by oestrogen deficiency (33,34). Therefore, regular audiometric checks should be performed referring patients to otorhinolaryngological departments.
As with other congenital conditions such as Kallmann syndrome, TS has been associated in medical literature with disordered olfactory and taste function. Unfortunately, this association is based on anecdotal observations, and the mechanisms responsible for the loss of smell are poorly understood. Considering smell and taste dysfunctions, only two studies have been published on TS patients. Nine TS women were found to have elevated detection and recognition thresholds to three odorants assessed, as well as sour and bitter thresholds using a taste test (35). Valkov published similar findings in 20 patients with TS eight years later (36).

Psychosocial development

Although there are exceptions, intelligence is within the average range in most females with TS, with good verbal skills. However, some have difficulties with non verbal learning disabilities, including number work, visuospatial and perceptual abilities and motor coordination. Moreover, parents report a reduced concentration span with less short-term memory. Again, the severity of the cognitive impairment is linked with the karyotype, being worse in pure monosomy than mosaic patterns (24). Differences in brain structure, including smaller parietal lobes, parietaloccipital and prefrontal volumes correlate with differences in brain functions (37).

Furthermore, women with TS have some personality traits, with greater difficulty in making friends and entering into sexual relationships. Although the difficulty in non verbal communication may have a role, poor self-image as a result of short statue, dysmorphology and delayed sexual maturation may be the main cause. Females with TS should have access to clinical psychologists for counselling related to anxieties and for working to improve specific areas of deficiency. Families should have support in obtaining appropriate therapy, including special accommodations at school (2).

Other disorders

Congenital renal disorders are 9-fold more common in women with TS than in the general population (5). These abnormalities include horseshoe kidney, duplex systems and rotated kidneys (Fig. 3). Malformations are more common in 45X0 monosomy females, being related to neither hypertension nor other clinical

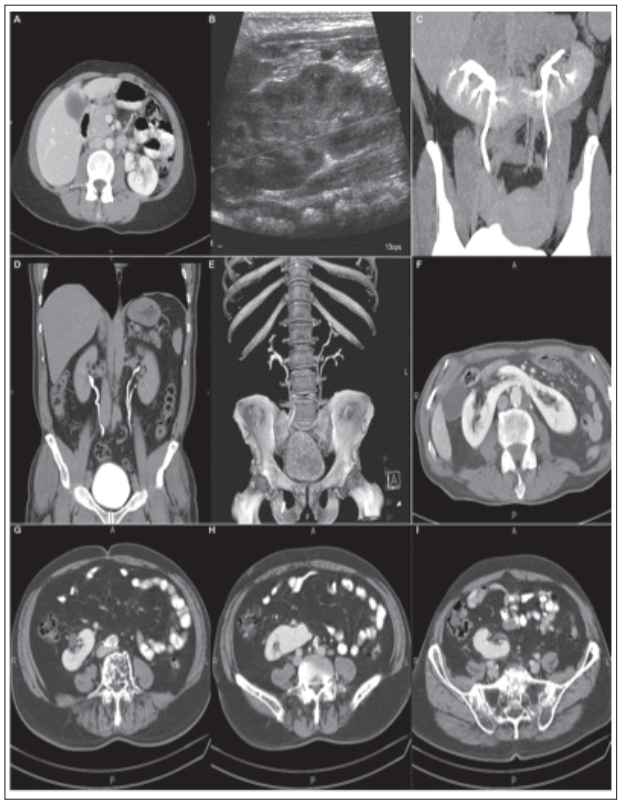

Fig. 3 - Main renal malformations associated with Turner's syndrome. (A) Axial plane of an abdominal TC showing a right renal agenesia. (B) Abdominal ultrasound showing a compensatory right renal hypertrophy in a newborn with unilateral agenesia. (C) Coronal CT-urography reconstruction showing a horseshoe kidney. (D) (E) Coronal CT-urography reconstructions showing a duplex system of the left kidney. (F) Axial plane of a CT showing a horseshoe kidney. (G), (H), (I). Three different axial CT planes of the same patient showing renal malposition, being both kidneys on the right side.

symptoms. Nevertheless, renal ultrasound is recommended at diagnosis and should be repeated at the time of adult transfer.
The most common ocular findings linked to TS are strabismus, ptosis and amblyopia. All patients should have eye assessment carried out during follow-up if required.

Conclusions

To conclude, females with TS have a high risk of developing medical problems, from infancy to adulthood. After early diagnosis, they should be followed by a multidisciplinary team of specialists to control the comorbidities associated with this syndrome (Table 3). At first, specialized paediatricians should perform a congenital malformation screening, with echocardiography and renal ultrasound. In this period, it is recommended to follow guidelines for the treatment with growth hormone, as well as oestrogen replacement for correct sexual development.
In adulthood, these females should be followed in specialized endocrinological-gynaecological units to diagnose disorders which debut in adulthood, such as metabolic, immunological and sensorineural disorders. Hormone replacement treatment should be guaranteed, as should reproductive and sexual advice.
Some studies from France (3), Belgium and the United States (38, 39) have reported that only a small minority of females (approximately 3.5%) with TS are transferred to an adult service that provides health assessment according to international guidelines. Several aspects of these recommendations are controversial: how to optimize screening evaluations, which cardiac malformations contraindicate assisted pregnancy, the best options for hormone replacement therapy and when to use them, or the evaluation of the influence of GH.
It should be highlighted that a small number of studies of TS patients in adult life have been published. Furthermore, gynaecologists follow TS women in adulthood and usually a lack of paediatric data exists, hindering patient's complete evaluation. Finally, patients from different ages have been managed following different protocols, due to the improvement of the prenatal or early diagnosis. This fact hampers the evaluation some studies included in the systematic review.

TABLE 3 - SUGGESTED FOLLOW-UP OF ADULT WOMEN WITH TURNER'S SYNDROME.

Baseline
Height, weight
Tanner staging
Blood pressure
Fasting glucose, insulin, lipid profile, hepatic profile
Thyroid profile, thyroid autoantibodies. Gonadotropins
Karyotype
Echocardiogram, renal and pelvic ultrasound. Electrocardiogram
Bone mineral density
Audiogram
Annual follow-up
Weight
Blood pressure
Fasting glucose, insulin, lipid profile, hepatic profile
Thyroid profile
3-5 yearly
Thyroid autoantibodies
Bone mineral density
Audiogram
Echocardiogram, electrocardiogram
Pelvic ultrasound

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Insulin sensitizer and inositol in the treatment of infertile PCOS patients

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Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in women and the most common cause of anovulatory infertility, affecting 5-10% of the population.

Infertility is the presenting problem for about 40% of PCOS patients. If pregnancy is achieved, other reproductive problems, such as miscarriage, emerge.

Approximately 60-70% of PCOS patients are obese, and it is well known that obesity is associated with insulin resistance. However, PCOS patients have evidence of insulin resistance beyond that of obese women in the general population. Most studies have shown that impaired insulin sensitivity is present without obesity; however, any degree of obesity further impairs insulin action.

Insulin resistance is defined as a pathological condition in which target cells fail to respond to ordinary levels of circulating insulin. At the molecular level, impaired insulin signaling results from mutations or posttranslational modifications of the insulin receptor or any of its downstream effectors' molecules. Insulin resistance could be accounted for by a defect in insulin binding to its receptor or to a shortage of insulin receptors; however, there is recent evidence to suggest that insulin resistance is most often due to a postbinding defect in insulin action.

Whatever the pathogenesis of insulin resistance, the resulting hyperinsulinemia is seen as a cause of the main features of PCOS, namely hyperandrogenism and anovulation. It was shown that the action of insulin on the ovaries is mediated by inositolglycan mediators and is therefore distinct from the insulin-activated tyrosine phosphate cascade that enhances glucose utilization. This indicates that the pathways of induction of insulin signaling are also separate in the ovaries and that the action of insulin on steroidogenesis is maintained even in cases of insulin resistance.

Hyperinsulinemia may increase androgen production in PCOS by stimulating the ovaries directly, or indirectly through stimulation of LH secretion and inhibition of IGF binding protein (IGFBP) and SHBG synthesis and secretion. Finally, insulin may also stimulate adrenal androgen secretion.

On the basis of the theory that insulin resistance and hyperinsulinemia may be a relevant contributor to the pathophysiology of PCOS, it has been hypothesized that insulin-lowering agents, by reducing hyperinsulinemia, might improve endocrine and reproductive abnormalities with PCOS (Table 1).

There is now a large body of data documenting the clinical efficacy of metformin in the treatment of PCOS-associated insulin resistance. Metformin is an "old" drug mainly used to lower blood sugar in NIDDM. Improvement in insulin resistance is generally accompanied by an improved metabolic profile, a significant reduction in hyperandrogenemia, and an improvement in ovarian function and menstrual cycles.

TABLE 1 - INSULIN-REDUCING ORAL DRUGS.

Oral antihyperglycemic drugs	
Solfonilureas	Chlorpropamide
	Tolbutamide
Biguanides	Phenformin (withdrawn for lactic acidosis)
	Metformin 500-850 mg, 1-3 times daily
Insulin resistance-reducing agents	
Thiazolidinediones (TZD)	Ciglitazone (no experience in PCOS)
	Pioglitazone 45 mg daily
	Troglitazone 600 mg daily
	Rosiglitazone 4 mg twice daily
	Ciglitazone (no experience in PCOS)

The first pharmacological approach to induction of ovulation in women with PCOS is clomiphene citrate. About 75% of patients ovulate in response to this drug, and most do so at a dose of 50 or 100 mg; the maximum dose is generally regarded as 250 mg/d. The number of nonresponders is therefore high. These women are defined as "clomiphene resistant" and are generally obese and more insulin resistant than responders. Because increasing obesity is associated with increasing hyperinsulinemia, the high degree of hyperinsulinemia in obese women with PCOS may account for their low responsiveness to clomiphene.

Presuming that the pathogenetic basis of clomiphene resistance was hyperinsulinemia, several authors investigated the effect of metformin therapy on the response of PCOS patients to induction of ovulation with clomiphene. Compared with placebo, metformin therapy resulted in a more than 10-fold increase in clomiphene-induced ovulation (90% of women ovulated).

It has been shown that the risk of ovarian hyperstimulation syndrome in PCOS increases with increasing insulin resistance. This again emphasizes the important role of insulin as follicle growth factor.

Based on this concept, a reduction in insulin resistance by means of metformin therapy was associated with a more "physiological" ovarian response to exogenous gonadotropins. Indeed, combined metformin-FSH therapy was followed by recruitment of fewer follicles and by lower estradiol levels at the moment of hCG administration. The percentage of cycles in which hCG was withheld because of excessive follicular development was therefore significantly lower in cycles treated with metformin.

In PCOS patients undergoing *in vitro* fertilization (IVF)-embryo transfer or intracytoplasmic sperm injection, combined metformin-gonadotropin treatment has been shown to be better than gonadotropin alone with recruitment of fewer follicles and lower plasma concentrations of estradiol.

Because metformin therapy improves spontaneous and clomiphene- or gonadotropin-induced ovulation and conception rates in women with PCOS, the safety of metformin administration during pregnancy has to be proven. It is classified as a category B drug, which means that *in vitro* studies did not demonstrate teratogenicity.

However, a recently published European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) Consensus that addressed the therapeutic challenges of women with infertility and PCOS has concluded that metformin, the most widely studied insulin-sensitizing drug in patients with PCOS, either alone or in combination with clomiphene citrate (CC), has no advantage in inducing ovulation in patients with PCOS, and should be restricted only to those patients with glucose intolerance.

In addition, a systematic review by Costello et al. demonstrated that although the co-administration of metformin to gonadotropin ovulation induction does not improve ovulation, pregnancy rate (PR), or live birth rate, it does consistently affect ovarian response during ovulation induction, with variable effects on the length of ovarian stimulation, total dose of FSH used, and peak serum E_2 level.

The new thiazolidinediones (rosiglitazone and pioglitazone) appear promising for the treatment of insulin resistance associated with PCOS. However, data are available only for troglitazone, a drug with high liver toxicity that was withdrawn from commerce in 2000. Rosiglitazone and pioglitazone are safer and have proved effective in improving insulin sensitivity. Rosiglitazone and pioglitazone are category C drugs that have been demonstrated to retard fetal development in animal studies. Because their use could restore ovulation and fertility, their presumed embryotoxicity would limit their use (Table 2).

The inositol phosphoglycans (IPGs) are putative mediators in a nonclassic insulin signaling cascade for glucose uptake and use. Insulin-resistant women with PCOS display decreased insulin-stimulated release of d-*chiro*-inositol (DCI)-containing IPGs (DCI-IPGs) during an oral glucose tolerance test (OGTT), compared with control women, which was related to impaired coupling between insulin action and the release of the DCI-IPG.

Oral nutritional supplementation with inositol, part of the vitamin B complex (B8) and an intracellular second messenger, was demonstrated to enhance insulin sensitivity and improve the clinical and hormonal characteristics of patients with PCOS. In addition, inositol supplementation was shown to restore spontaneous ovulation with the consequent increase in conception, either alone or when combined with gonadotropin.

Inositol nutritional supplementation to insulin-resistant patients with PCOS, during a low-dose go-

TABLE 2 - MECHANISM OF ACTION AND SIDE EFFECTS OF OTHER INSULIN SENSITIZING DRUGS.

Drug	Mechanism of action	Side effects
Diazoxide	Insulin secretion	Hyperglicemia, salt retention, hyperuricemia, hot flushes, palpitations
Acarbose	Carbohydrate digestion	Abdominal discomfort
Somatostatin analogs	Insulin secretion	Abdominal discomfort
D-*chiro*-inositol	Insulin post-binding signaling	Not reported

nadotropin step-down ovulation induction regimen, produces very good clinical results with a significant reduction in cancellation rate and the consequent improvement in clinical PR.

These observations are in accordance with the well-established beneficial effects of inositol on reducing insulin resistance, improving ovarian function, and decreasing hyperandrogenism, which are comparable with those of metformin. Therefore, not surprisingly, by adding the inositol nutritional supplementation we were able to achieve a more efficient and less vigorous ovarian response to gonadotropin. This more delicate response, as reflected by the significant decrease in the number of growing follicles, results in the consequent decrease in cancellation rate and improved PR.

Although the systematic review on metformin co-administration during gonadotropin ovulation induction could not demonstrate any significant improvement in ovulation or PRs, metformin intake is associated with a significantly high incidence of gastrointestinal disturbance, and its safety throughout pregnancy is not yet established (category B drug, according to the Food and Drug Administration). On the other hand, inositol is a nutritional supplementation, its intake is not associated with any significant side effects, and its co-administration with gonadotropin resulted in less vigorous ovarian response and the consequent decrease in cancellation rate and improved PR.

In summary, adding the inositol nutritional supplementation during the low-dose gonadotropin ovulation induction seems to considerably diminish the chance of multifollicular development and its consequences. An added value of this protocol is that it has a very low cycle cancellation rate and produces very good clinical results with an excellent safety profile.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Prevention and early diagnosis of gynaecological malignancies in Hereditary Non-Polyposis Cancer (or Lynch) Syndrome

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Background

Women with Hereditary Non-Polyposis Cancer Syndrome (HNPCC), also known as Lynch syndrome, are carriers of a germline mutation in one of the mismatch repair (MMR) genes MLH1, MSH2 and MSH6. Their life-time risk of endometrial and colorectal carcinoma is 25-70% and that of ovarian carcinoma it is approximately 10%. There is also a chance of more than 10-25% of developing tumors of small intestine, stomach, renal pelvis, ureter and central nervous system (1).
Even the cancer risk of women with Lynch syndrome is so high, on the basis of the available limited literature, there are not enough specific studies on primary prevention and any strict advice for or against surveillance for gynecologic cancers is not evidence based (2).
Most of the studies that form the basis for the guideline recommendations are descriptive and/or retrospective in nature, many of them were based on expert opinion (3). Being a rare disease it is unlikely that adequate powered randomized controlled studies will be available in the next future thus we tried to use the existing literature to help this patients prevent gynecologic cancers and diagnose them as early as possible. A systematic literature search using Pubmed and the Cochrane Database of Systematic Reviews, reference lists of retrieved articles and manual searches of relevant articles was performed. We added our experience of an ongoing specific study at our institute approved by the Ethics Committee.

Primary prevention of gynaecologic cancers

Endometrial and ovarian cancer in the general population are preventable neoplasm because the majority of risk and protective factors are modifiable: body weight, diet, physical exercise,... Obesity increases the risk while a diet high in fruits and vegetables may lower the risk. Physical activity is an understudied but promising cancer preventive strategy (4). There is evidence that the expression of Lynch syndrome is also influenced by environmental factors. The genes known to be involved are MSH2 and MSH6 and the protein products of these genes help to repair mistakes made in DNA replication. If they are mutated, the replication mistakes are not repaired, leading to damaged DNA and cancer. Dietary and lifestyle factors could thus theoretically modify the risk, but there are few studies on primary prevention of female cancers specifically for the Lynch syndrome that confirm that these high risk patients need to follow the cancer prevention strategies than the general population. The CAPP studies prove protection for colorectal cancer only. In the CAPP2 randomised trial, carriers of Lynch syndrome had a HR of colorectal cancer of 0·63 (95% CI 0·35-1·13, p=0·12) with 600 mg aspirin per day for a mean of 25 months (5).

Endometrial cancer (EC)

Women with Lynch syndrome (HNPCC) have a high risk of developing endometrial cancer (EC):

up to 70% lifetime risk (43-71%) with an average +1-2% annual risk in 35-65 age group. EC risk is about seventy times more than in the general population.

Early diagnosis

The peak EC incidence mean age is 49y (45-50y) when symptom detection could be more difficult. Starting age for surveillance of 30-35 years is therefore advised. On the basis of literature available, no advice can be provided regarding the age at which screening should finish. The five-year survival after treatment of endometrial carcinoma with Lynch syndrome is high, as it is in sporadic cases, namely >80%.

The efficacy of surveillance by the recommended annual o biannual transvaginal sonography (TVS) with endometrial biopsy (EB) from 35y age is not established. The efficacy of annual endometrial biopsy is higher than exclusively transvaginal ultrasonography. Annual surveillance with gynaecological examination, transvaginal ultrasonography and determination of the serum tumour marker CA125 in 826 patient years did not yield any pathological findings in a first study (6).

An endometrial carcinoma was found in two women of the previous study during the interval between two surveillance visits, 6 and 24 months after the last ultrasound, because they had vaginal bleeding (7). In another cohort study (8) three premalignant lesions of the endometrium were detected during transvaginal ultrasonography. The study period included a total of 197 patient years at risk. One patient developed a symptomatic interval carcinoma of the endometrium, stage IIA.

Ovarian pathology was not detected in either of the above mentionend cohort studies. In a third study on the efficacy of gynaecological surveillance (9) not only transvaginal ultrasonography but also endometrial biopsy was performed. Out of 175 mutation carriers, 759 patient years were evaluated. Four of the 14 diagnosed endometrial carcinomas were found by transvaginal ultrasonography. Eight of the 14 by endometrial biopsy, which also detected a potential premalignant hyperplasia of the endometrium in 14 patients. Four cases of ovarian carcinoma occurred during this period, but none of these were detected during surveillance. In conclusion, two of the three available studies suggested that surveillance may lead to the detection of premalignant lesions, and one study suggested that it may also lead to the detection of endometrial cancer at an early stage. More prospective studies are needed to establish the most appropriate screening protocol.

Guidelines on endometrial surveillance

IKNL Guideline 2009, Level 3 (1)
(Annual) surveillance by transvaginal ultrasonography may detect premalignant lesions of the endometrium.
Surveillance is more effective when endometrial biopsy is also performed.
Despite annual surveillance by transvaginal ultrasonography with or without endometrial biopsy, interval endometrial carcinomas still occur. None of the studies, till now, showes that surveillance improves survival.

Vasen Giudeline 2007, Level 3 (3)
Surveillance by gynaecological examination, transvaginal ultrasound and endometrial biopsy starting from age 30-35 years may lead to the detection of premalignant lesions and early cancers.
Prophylactic hysterectomy and salpingo-oophorectomy may be an option for women with Lynch syndrome, since it substantially reduces site-specific cancers.

LNG IUS (Mirena®)

EC in Lynch syndrome is oestrogen-driven and progestogens reduce endometrial hyperplasia and EC risks. Thus when pregnancy is no more need the intrauterine levonorgestrel system should be the first choice for reduction of atypical endometrial hyperplasia (AEH) and endometrial cancer in Lynch syndrome. The high dose of levonorgestrel (20mcg per day) locally released by Mirena® reduces endometrial hyperplasia and it causes the development of a thin inactive endometrium. It has also been documented to reduce or reverse atypical hyperplasia and EC development. This IUS is a widely-used and well-tolerated contraceptive and it protects for four years from endometrial hyperplasia in women using oestrogen replacement therapy (10,11). The POET trial (Prevention of Endometrial Tumours) is being developed to explore the possibility of chemoprevention using the progesterone-releasing Mirena® intrauterine device for 4 years in reducing the development of atypical endometrial hyperplasia and carcinoma in women with hereditary Non-Polyposis Colorectal Cancer (HNPCC, Lynch Syndrome) aged 35-65y. This trial was approved by or ethics committee and it is ongoing in our institute. At the first visit women have a full gynaecological evaluation with transvaginal ultrasound or hysteroscopy, endometrial biopsy, and full menstrual history. Those with complex atypical hyperplasia or cancer will be treated as clinically indicated. Women with normal endometrium or simple or complex e non-atypical

endometrial hyperplasia are randomised for Mirena® insertion or only surveillance. Mirena® insertion is till now well accepted and tolerated. Patients are followed with annual menstrual history, transvaginal ultrasound and endometrial biopsy. If biopsy is inadequate and/or endometrial thickness sufficient a hysteroscopic biopsy is performed. We are following seven patients because this syndrome is rare and this prevents any statistically significant conclusion about efficacy on EC by now. We did not observe any side effect or development endometrial hyperplasia with Mirena®.

Ovarian cancer (OC)

Lynch syndrome patients have a lifetime risk of ovarian cancer of 8-10% (versus a population risk of 1.45%), with younger age at diagnosis: mean 43y vs 59y. The combination of ultrasound with Ca 125 and new markers like HE4 could improve early diagnosis of OC and even EC which is particularly challenging in this high risk population. Ovarian surveillance effectiveness in Lynch syndrome carriers is not to be expected, at least on the basis of the most extensive literature regarding the efficacy of this surveillance in BRCA1/2 mutation carriers.

Prophylactic surgery

The possibility of preventative hysterectomy, including both adnexa, should be discussed, particularly in the case of a laparotomy in a MMR mutation carrier after child bearing age. Prospective studies regarding the effects of prophylactic surgery on the incidence of, and mortality as a result of, gynaecological tumours with Lynch syndrome and the negative effects of surgical intervention are lacking.
A retrospective study proposed preventative surgery (hysterectomy, adnectomy) in MMR mutation carriers to prevent endometrial and ovarian carcinoma. In a cohort of 315 women, all mutation carriers, 61 of whom had prophylactic surgery and were then followed up for approximately 10 years. No endometrial cancer or ovarian cancer developed in those women who had prophylactic surgery, whereas 33% of women who did not have surgery developed endometrial cancer and 5.5% developed ovarian cancer (12). Patients candidates for prophylactic hystero-adnexectomy should be informed of the limits of our current knowledge on the benefit/ risk balance.

Transvaginal ultrasound plus Ca 125 and HE4

Transvaginal ultrasound is the most effective, economical, not invasive way to early diagnose endometrial and ovarian cancers, but it is not accurate enough to be sufficient ad the only screening tool for genital cancers in Lynch syndrome. HE4 is a new promising cancer marker that could be included in the gynecologic screening because it improves ovarian cancer sensitivity, if it is combined with Ca 125, more than magnetic resonance imaging (13). HE4 is more specific to discriminate between endometriosis and ovarian cancer (14). HE4 it is also more sensitive in early-stage endometrial cancer compared to Ca 125 (15).

Hormonal contraceptives

Use of oral contraceptives significantly reduces the risk of development of endometrial and ovarian carcinoma for decades. The protective effect increases with the length of time they are used in the general population (16). Studies have not been specifically performed in patients with Lynch syndrome but hormonal contraceptives have many other benefits and an overall good benefit/risk ratio.

Conclusion and proposal of a screening protocol for gynecological cancers

Women with a genetic predisposition to Lynch syndrome need to be well informed about their risk of endometrial and ovarian carcinoma The consultation with the gynecologist may also contribute to reduce endometrial and ovarian cancer by improving lifestyle in order to act on the main modifiable risk and protective factors. The same advices could help to improve the overall health.
Even though it is not jet demonstrated that it improves survival, gynecologic periodical controls can help Lynch syndrome patients lighten the psychological burden of the knowledge of the high risk of gynecological malignancies. Every patient must be specifically alerted to recognize the early symptoms of endometrial carcinoma, considering any even light abnormal vaginal blood loss, leading to a greater chance that endometrial carcinoma of a low stage is detected.
Lynch syndrome patients must be made aware of the real possibilities and limitations of surveillance. In order to overcome the poor results of the existing screening guidelines we propose and open to discussion the following improvements.

A six month gynecologic control versus the standard annual is better for this high risk cancer patients because of the following reasons:

1. the annual o or biannual screening did not demonstrate to improve survival;
2. all the gynecologic cancers were reported in that interval;
3. endometrial and ovarian cancers have a fast growing rate;
4. the gynecologic cancer risk is high enough to justify that.

An annual hysteroscopy and/or sonohysterography in the perimenopausal period and or in selected higher risk cases is better than a blind endometrial biopsy because:

1. random or blind endometrial biopsy is not enough to detect focal endometrial pathology;
2. most of endometrial cancers in Lynch syndrome patients appear at perimenopausal age (mean 49 years) where bleeding symptoms and endometrial thickness are unreliable early detection strategies.

HE4 could be proposed as a new promising cancer marker that could be included in the gynecologic screening strategies in Lynch cancer patients for the following reasons:

1. it improves ovarian cancer sensibility if it is combined with Ca 125 and transvaginal ultrasound;
2. it is more specific to discriminate between endometriosis and ovarian cancer;
3. it is more sensitive in early-stage endometrial cancer compared to Ca 125.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Meeting the needs of adolescents with endometriosis

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Introduction

Dysmenorrhea is one of the most common complaints in adolescence, but it is frequently underevaluated and consequently undertreated. Despite endometriosis has long been considered a disease of adult women, studies reveal that endometriosis rates in adolescent patients undergoing diagnostic laparoscopy for secondary dysmenorrhea and chronic pelvic pain evaluation, range from 19% to 75% (1-4). Most adult patients report onset of symptoms even from adolescence. Statistically, the diagnosis is established after a mean of 9.28 years of the onset of symptoms (5-7). Endometriosis has also been associated with the presence of uterovaginal anomalies, such as complete transverse vaginal septum, imperforate hymen and cervical agenesis, which in the majority of cases, are diagnosed in adolescence (8,9).

Clinical presentation

Unlike women of reproductive age with endometriosis,-in which subfertility is the main problem- in adolescents the main symptom that leads them to seek medical advice is pelvic pain. In most cases (62.6%), pain is both cyclic and acyclic, while presenting with acyclic or cyclic pain alone is rare. Other complaints include: dyspareunia, gastrointestinal or urinary symptoms, menstrual disorders and vaginal discharge (2). In many cases adolescents' ability to attend and perform in school or to participate in social activities can be impaired, leading to major psychological consequences. Moreover, failure to control pelvic pain may lead to negative consequences in relationships with the young girl's parents, in body image and in self-esteem.

Evaluation of the patient

Initial investigation of the patient should include a thorough medical history with specific questions about the frequency and the duration of the pain, and its association with bowel, bladder or sexual function. Inquiries regarding familial history of endometriosis should not be omitted, as studies have shown that there might be a genetic predisposition for endometriosis, but this is not a prerequisite for the development of the disease (10).
Physical examination should begin with the inspection of the external genitalia, and the insertion of a cotton-tipped swab through the hymen, in the vaginal canal in order to rule out the presence of imperforate hymen, low vaginal septum or any other obstructive Müllerian duct anomaly. Clinical findings in adolescents with endometriosis are different from those of adults, representing the different stage of the disease. In adults the uterus is fixed and retroflexed due to adhesions, while in adolescents only mild tenderness might be evoked during palpation/bimanual examination. Findings from the ovaries are also rare in this age group, as endometriotic cysts are found more often in adults.
Ultrasound imaging is useful in the diagnosis of endometriotic cysts or in cases of deep infiltrating endometriosis, which is rare in this age group. Magnetic Resonance Imaging (MRI) can be helpful in detecting reproductive tract anomalies. Finally, CA-125 is a very sensitive marker of endometriosis but its low specificity makes it inappropriate as a diagnostic tool.
The definitive diagnosis can only be set through laparoscopy and biopsy. Staging of the disease should be made according to the guidelines proposed by the American Society for Reproductive Medicine (ASRM).

Unlike adult patients who, in most of the cases, have the classic black/grey "powder burn" endometriotic implants, adolescents have stage I (77-92%) or stage II (8-22%) disease with red, white or clear lesions. Patients with endometriosis due to outflow obstruction tend to have more extended disease, but remittance is established as soon as the anatomic anomaly is surgically corrected (11,12).

Treatment of endometriosis in adolescence

Treatment of adolescents with endometriosis should be focused to: symptom's relief, the supression of disease progression and preservation of future fertility. Options include:

Non-steroidal anti-inflammatory drugs (NSAIDs)
As early stage endometriotic lesions are documented to secrete prostaglandins, NSAIDs can provide pain relief in this age group (13,14).

Oral Contraceptive Pills (OCPs)
OCPs are the first line treatment for endometriosis in adolescence. OCPs have a very low adverse effect profile and are considered safe for long time treatments. They inhibit endometriotic implant growth by inducing a hypoestrogenic environment, and also alleviate from luteal-phase symptoms of endometriosis by inhibiting ovulation. Continuous use of OCPs is suggested, despite the possibility of breakthrough bleeding (15-17).

GnRH agonists (GnRHa)
GnRHa cause a reversible suppression of the hypothalamic-pituitary-ovian axis, thus controlling growth and bleeding of endometriotic implants. Treatment with GnRHa has been associated with adverse effects on patients' peak Bone Mass Density (BMD) accumulation. It is recommended that GnRHa should not be administered in adolescents younger than 16 years old. Moreover the U.S. Food and Drug Administration (FDA) has not approved its use for therapy lasting longer than six consecutive months. Add-back therapy with norethindrone acetate or with combination of estrogens combined with medroxyprogsterone acetate, can help reducing the adverse effects associated with the administration of GnRHa and minimize bone density loss (18-22).

Depot Medroxyprogesterone Acetate (DMPA)
Progestogen-only therapy acts by suppressing gonadotropins and causing decidualisation, necrosis and absorption of functional endometrial implants. Although it has been proved be effective, there are concerns regarding its negative effect on bone mineralization. It remains a relatively safe alternative therapeutic scheme for adolescents who have contraindications or objections against continuous use of oral contraceptive agents (23).

Danazol
Danazol creates a hypoestrogenic environment, inducing endometrial atrophy, while also raising androgen levels and having direct androgenic action. Although it can provide adequate relief from symptoms, its adverse effects (weight gain, bloating, decreased breast size, acne, oily skin and hirsutism), make it an unpopular therapeutic option for adolescents (24,25).

Surgical treatment
Surgical management has also been proved to be effective treatment for the disease associated pain in patients with endometriosis (26). Radical procedures like oophorectomy and hysterectomy have no place in adolescence, even in patients with severe endometriosis, in order to preserve fertility. Laparoscopy is generally preferred. Special knowledge and experience are mandatory for the surgeon, who must be familiar with the appearance of early stage endometriotic lesions that are commonly found in adolescents. As with surgical intervention only the visible endometriotic lesions of the pelvis and the abdomen are removed, while all the factors (hormonal, genetic and physiologic) that predispose to endometriosis remain unaltered, recurrence of endometriosis is certain. As a result, postoperative hormonal treatment is imperative in order to reduce the possibility of recurrence.

Conclusions

Endometriosis is a condition affecting both adult women and adolescents, but there are significant differences in the characteristics of the disease between these groups. Adolescents usually present in a less advanced stage with early atypical lesions, while adult women tend to have classic "powder burn" lesions and a more advanced stage of the disease. Treatment also differs between these two groups, as the necessity of fertility preservation and the immaturity of the skeletal system, dictates the use of different therapeutic schemes. During adolescence, endometriosis should be diagnosed as soon as possible, so as to avoid the physical and psychological sequalae of the disease. As it is a chronic progressive disease, psychological support should be provided to the girl.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Ovary and senium

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Introduction

Aging is a process that involves structural and functional alterations in all organs of the human body. The aging of gonads, to be precise, represents a particular case, because these organs are not exactly essential for the function of the rest of the body.
For what concerns women, menopause is the final step in the process defined *ovarian aging*, which is directly related to the exhaustion of ovarian follicles. Testicular endocrine function, in contrast, begins during embryonic stages, is interrupted between birth and puberty, and continues from then on, along with spermatogenesis, with only slight decline in the old age (1).
The current knowledge of female reproductive aging has wide limitations: we are aware of the crucial role of ovarian changes in this process, where the continuous decrease in the number of follicles present in the ovarian cortex and their final depletion dictate the onset of cycle irregularity and the final cessation of menstrual cycle. As a matter of fact, when a woman reaches menopause, her follicular supply consists of about a thousand or less follicles, a number that is insufficient to sustain the cyclic hormonal variations necessary for menstrual cycle (2). Moreover, most of the numerous theories trying to explain aging highlight the concept that age-related dysfunctions might, at least partially, come from all the irreversible damages that physiologically accumulate during a cell's lifespan, as an unavoidable side effect of normal metabolism (3).
Endocrine changes are triggered by the decline in the negative ovarian feedback at the hypothalamo-pituitary unit, which causes alterations in the neuroendocrine control of cyclic ovarian function, such as the increase in serum FSH levels in the early follicular phase (4-5). This event may accelerate the depletion of the ovarian follicular reserve (6), by selecting ovarian follicles that would otherwise be excluded, at the expense of fertility (7) However, FSH increase begins late in the series of events that characterize aging, so it cannot be the sole responsible of the decline of oocyte quality associated with age. Even though the process of loss of follicles in the ovary begins at a relatively early stage of life, it is only when cycles lose its regularity that women notice the signs of the ongoing reduction of reproductive function.

Current knowledge and future challenges

Several studies in literature show that the number of follicles declines at a steady rate of 1000 follicles per month, from the mean age of 30 years onward, with large individual variation (8,9). Obviously, hormonal changes are not sufficient to explain the many-sided process of ovarian aging, especially because of their late occurrence. Thus, these variations may relate to possible differences between genetic preprogramming of germ cells during foetal life, damage of oocytes in the course of a woman's life, aberrant function of individual "meiotic clock", or changes in the functionality of the granulosa cells surrounding the oocyte. The role of the somatic compartment in the process of follicular aging has been highlighted by studies showing the ability of aged cumulus cells to facilitate the activation of apoptosis in the oocytes by means of specific factors such as ceramide, a sphingolipid second messenger involved in cellular senescence (10,11).
Thus, female *reproductive aging* is defined as a process

that increasingly reduces monthly fecundity (the ability to have a viable embryo implanted) after the age of 30 years (8).
The parallel decline in oocyte quality, next to quantity, is expressed by the observation of an increasing risk of aneuploidy and spontaneous miscarriages, and contributes to the gradual decline in female fertility with aging and the final occurrence of natural sterility (8,12). Recent researches have focused on the concept that oocytes coming from follicles selected in advanced reproductive age own reduced chances of achieving a level of nuclear and cytoplasmic maturation that, along with their increased apoptotic potential, make them less suitable for fertilization and subsequent embryo development (13).
The declining cohort of antral follicles with age firstly leads to a gradual elevation of FSH levels (14). At the same time, the gradual decline in the number and function of granulose cells of the antral follicle cohort is best represented by decreasing levels of anti-Müllerian hormone (AMH). This endocrine factor, mostly expressed by granulosa cells of primordial growing follicle pool, is considered a sensitive marker of ovarian reserve and, probably, of the earlier steps of aging (15,16).
Antral follicle count (AFC), last but not least, represents the imaging corresponding of the remaining pool of antral follicles in the ovarian cortex, and it is evaluated by ultrasound, with a transvaginal probe.
AFC and AMH are considered the best markers of the ovarian reserve, but their predictive value is limited, and the true impact of routine ovarian reserve testing (ORT) on the clinical management of ovarian aging remains to be demonstrated (17).
Development of reliable tests for the identification of women with a reduced reproductive life span is likely to come from combining endocrine, imaging and, especially in view of the high heritability of age at menopause, genetic informations. Recent studies have identified several interesting loci of small genetic variation that may determine foetal follicle pool development and subsequent decrease of this pool over time.
Furthermore, recent observations in both animal models and humans have demonstrated that aging may impede the collection of maternal RNAs required for the normal physiology of the oocyte, and subsequently used during early embryonic stages (18). The different genes expressed are involved in mitochondrial function and in the assembly of an efficient antioxidant enzymatic defence, as well as in a variety of functional processes, including cell cycle control, cytoskeletal structure, metabolic pathways, transcription regulation and stress responses (19).
However, at present it is generally accepted that aging is a result of both inborn and environmental factors (20). In fact, many environmental and life style factors have been suggested to affect age at natural menopause, but only cigarette smoking has been unequivocally proven to be related with the age at menopause. However, there is no single factor that consistently explains the variation in menopausal age, and, in short, environmental factors have been estimated to play only a minimal role in the process.
At the same time, the follicular microenvironment is considered extremely important for ovarian aging. The availability of an adequate vascular support, represented specifically by a sufficient ingrowth of capillaries into the theca, is essential for maintaining the correct physiology of follicular growth and selection (21,22). In this respect, it has been reported that an impaired vascularization may have crucial effects on follicle depletion, leading to reduced oxygen, hormonal and nutritional supply to the leading follicle. Reduced oxygen amount might ultimately trigger a condition of oxidative stress, even if knowledge of the exact role of ROS in ovarian aging is still conflicting (13). Despite the fact that the reasons and the mechanisms of potential age-related decline in ovarian follicle vascularity need still to be clarified, the presence of elevated levels of VEGF along with reduced blood flow in the follicular microenvironment of aging ovaries suggests that, in order to compensate for increasing hypoxia, granulosa and theca cells begin to produce VEGF, which nevertheless apparently fails in its attempt (24).
Another important aspect of ovarian aging is that the older a woman is, the higher her risk for developing ovarian cancer (21). The majority of sporadic ovarian cancers are diagnosed in women after menopause, although women with genetic or familiar risk factors tend to get ovarian cancer at a slightly younger age. Only the 10-20% of ovarian cancers is diagnosed in women under age 40: as a consequence, it seems clearly ever more important to follow women in this period of life for their reproductive health (25).
Thus, improved knowledge of the ovarian aging process may ultimately provide strategies for prediction of menopause and manipulation of the early steps of follicles selection for cancer prevention, contraception purpose and fertility lifespan lengthening ambitions. By now, we can only suppose that ovarian aging may be the product of the prolonged exposure of follicles to subtle oxidative damage, which irreversibly accumulate during a woman's reproductive life, gradually affecting vascular supply and promoting the accumulation of ROS in the ovarian microenvironment (19).
The real issue for ORT lies in the capacity of identifying women with a shorten fertility life span at such an early stage that adequate measures can be adopted. As menopause and the preceding decline in fertility seem to be related by a fixed time interval, if age at menopause could be adequately predicted, then any

young woman might be tested for her reproductive expectations.

By now, we appreciate that poor response to gonadotropin stimulation in assisted reproductive technologies (ART) is associated with a shorten reproductive lifespan. Our present possibility consists in combining reproductive outcomes at ART and other parameters with genetic tests and possible lifestyle modifications (such as weight reduction, smoking cessation) to make an assessment of the individual reproductive aging and reproductive expectations (26). In the future, the study of ovarian aging should deal with a multidisciplinary approach, with a strong interaction between biological and clinical research, leading to an elucidation of the effect of aging on ovarian and follicular vascularization and to a clear explanation of the role of oxidative stress in the pathophysiology of the female gametes.

Conclusions

In conclusion, the need the for an efficient and safe treatment of menopausal symptoms represents a great challenge to the healthcare system (27). Nevertheless, the decline in female fertility that takes place about 10-15 years earlier is less obvious and more complicated to predict, and ART cannot completely replace female reproductive function in many instances of reduced fertility (28).

With the modern trend of Western countries of postponing pregnancies often in the late 30s, the importance of understanding the onset of ovarian senescence and its regulation is becoming increasingly important: notwithstanding the recent progresses of science, the challenge is still on.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Treatment of Polycystic Ovary Syndrome (PCOS) in non-obese adolescents

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PCOS is a common endocrinopathy that affects about 5-10% of women of reproductive age, irrespective of the ethnic background, and typically manifests during adolescence. It is classically characterized by features of anovulation (amenorrhea, oligomenorrhea, menstrual irregularity) combined with symptoms of androgen excess (hirsutism, acne, alopecia) (1-3).

Over the past years, research has supported the key role of insulin resistance and associated hyperinsulinemia in the pathogenesis of PCOS and thus, in the development of its associated metabolic co-morbidities, including dyslipidemia, the metabolic syndrome, type 2 diabetes and cardiovascular disease, as well as reproductive co-morbidities, such as gestational diabetes mellitus and pre-eclampsia (4-6). Accordingly, PCOS is nowadays viewed as a variant of the metabolic syndrome (7) and interventions aim at improving insulin sensitivity, at attenuating visceral adiposity, low-grade inflammation, dyslipidemia and dysadipocytokinemia, and at lowering carotid intima-media thickness (IMT).

In non-obese adolescents with androgen excess and without pregnancy risk, a low-dose combination of flutamide (Flu, an androgen-receptor blocker) and metformin (Met, an insulin-sensitizer) proved superior to a drospirenone-oral contraceptive (OC) in correcting the endocrine-metabolic and body-composition anomalies (8). In young PCOS women receiving OCs, the addition of low-dose pioglitazone (Pio, 7.5 mg/d) to the FluMet combination further increased lean body mass and circulating HMW-adiponectin, and further reduced IMT (9).

Recently, we compared the effects of low-dose PioFluMet to those of an OC containing ethinylestradiol-cyproteroneacetate. Both reduced the androgen excess similarly, but had divergent effects on low-grade inflammation, on adipokines and on body adiposity, all of those effects being to the advantage of PioFluMet (Fig. 1) (10).

Genetic variants appear to influence the response to therapy; more specifically, they may modulate the efficacy of metformin. For example, genetic polymorphisms in the serine-threonine-kinase (*STK11*) that catalyses the activation of AMPK in the liver and in the gene encoding for the organic cation transporter 1 (*OCT1*), and the length of the pentanucleotide repeat in the sex hormone-binding globulin (*SHBG)* gene and of the *CAG* repeat in the androgen receptor gene (11).

In conclusion, a novel low-dose combination of insulin sensitizers plus an anti-androgen holds potential as a pathophysiology-based treatment of androgen excess in young girls. Such combination may avoid that those young girls, who are not at risk of pregnancy, are nevertheless exposed to supraphysiological doses of estrogen. The efficacy and safety of PioFluMet remain to be studied over the longer term and in larger cohorts.

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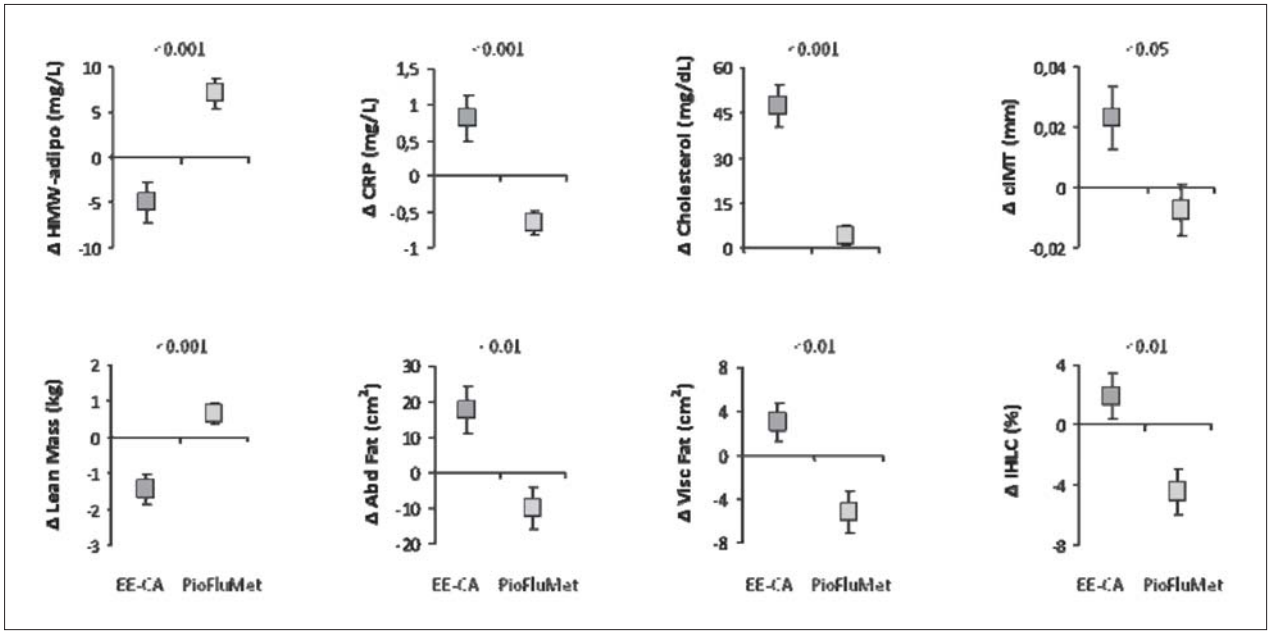

Fig. 1 - Changes over 6 months in HMW-adiponectin (HMW-adipo), C-reactive protein (CRP), total cholesterol, carotid intima-media thickness (cIMT), lean mass, abdominal and visceral fat areas (Abd Fat and Visc Fat, by MRI) and intrahepatic lipid content (IHLC, by MRI) in adolescent girls with androgen excess treated with ethinylestradiol-cyproteroneacetate (EE-CA, N=17; orange) or low-dose pioglitazone-flutamide-metformin (PioFluMet, N=17; green). Results are shown as mean and SEM.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Stem cells in endometrium and in endometriosis

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The concept of adult stem cells mediating cyclic endometrial regeneration is increasingly accepted. About 2% of endometrial cells display the ‚side population'-phenotype, a surrogate stem cell marker based on the expression of multidrug-resistance-proteins such as MDR1 or ABCG2 (1). Additional evidence for stem cell activity is provided by the expression of stem cell markers in the endometrium, including the pluripotency-associated transcription factors OCT-4, SOX2, NANOG and KLF4, of mesenchymal (CD73, CD90, CD105, CD146) and endothelial progenitor cell markers (CD31, CD34, CD144) as well as additional adult stem cell markers such as Notch-1 and Msi1 (2-5). Endometrial stem cells have been isolated and differentiated in vitro into cell types of osteogenic, adipogenic, chondrogenic, and myogenic lineages, demonstrating high developmental plasticity resembling mesenchymal-stem-cell like properties (6-9) (Fig. 1). In Xenograft-studies, these cells were capable of generating endometrial tissue in vivo (1,2,8). At least a part of endometrial stem cells may originate from the bone marrow, as demonstrated by reconstitution experiments in humans and mice (10-12). However, it is not clear if these cells are the sole source of endometrial stem cells, as different stem cell pools appear to exist, based on differences in surface marker expression and clonal colony formation assays (1-9). Although the basalis may be the main location of endometrial stem cells, these cells can also be obtained from more superficial layers by low invasive techniques and from menstrual blood, opening new diagnostic and therapeutic perspectives (2,3,9,13,14). From an endocrinological perspective it is noteworthy that endometrial stem cells express no or very little estrogen receptors. This has been demonstrated for slow cycling murine label retaining cells (15,16), for human

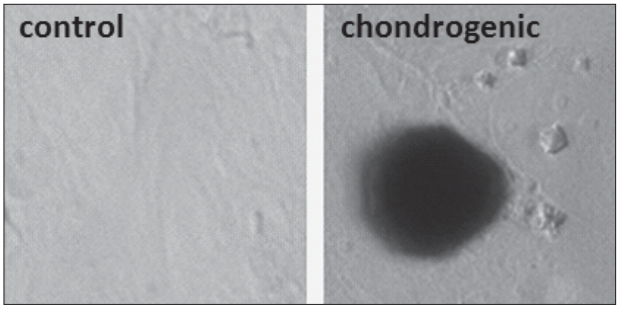

Fig. 1.

endometrial side population cells (2) and for human endometrial mesenchymal stem cells (17). Nevertheless, indirect estrogenic stimulation appears to occur via estrogen-receptor positive cells in the stem cell niche, which may secrete cytokines capable of modulating stem cell behaviour in an estrogen-dependent manner (15,16).

Several lines of evidence suggest an involvement of dysregulated stem cell activity in the pathogenesis of endometriosis. Besides the presence of stem cells in menstrual blood (13,14), which may be ectopically distributed by retrograde menstruation, the monoclonal origin of some endometriotic lesions (18) and the long-term culturing properties of cells derived from endometriotic lesions (19) served as early indicators for the potential presence of stem cells in endometriosis. Noteably, increased numbers of putative endometrial stem cells expressing the stem cell markers Msi1, Sox2, Oct4 and c-kit have been detected in endometriotic tissue compared to healthy endometrium, suggesting an involvement of stem cells in the pathogenetic process (3,5,20). At the functional level, it could be demonstrated that ectopic endometrial

mesenchymal stem cells display invasive behaviour both in vitro and in a mouse model (21). The dysregulated stem cell activity in endometriosis suggests that an induced differentiation of stem cells may be a promising therapeutic approach in addition to the use of stem cell markers for diagnostic purposes (3,22). Moreover, therapeutic applications utilizing endometrial stem cells reach far beyond the field of Gynecology and Obstetrics: The high endometrial expression levels of pluripotency factors (5) facilitate the generation of induced pluripotent stem cells from endometrial tissues (23,24). Overall, research on endometrial stem cells forms a base for the development of novel therapeutic approaches including an in vitro differentiation of endometrial stem cells into contractile cardiomyocyte-like cells (13), dopaminergic neurons (25) or insulin-producing cells (26,27), which have already been successfully applied in animal models of myocardial infarction, Parkinson's disease and diabetes, respectively. The encouraging data generated in these experimental disease models provide novel perspectives for the future treatment of female patients with autologous endometrial stem cell-derived regenerative cells, overcoming the hindrance of immune rejection associated with heterologous cellular treatment approaches.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The role of progesterone and thyroid hormone in trophoblast invasion in early placental development

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Introduction

Placental tissues contain a heterogeneous population of cells, including villous cytotrophoblasts, syncytiotrophoblasts and extravillous trophoblasts (EVTs). The human early placenta is characterized by the invasion of EVTs to the decidua, leading to direct contact between EVTs and maternal blood. EVTs are mainly uninuclear cells comprising all the trophoblastic elements located outside the villi. EVT has two distinct phenotypes, proliferative and invasive. The activity of the invasive EVTs is dependent on its apoptotic capacity and less on its proliferative potential. Apoptosis is an important determinant in regulating placental growth. Actually, apoptosis is more evident in the invasive EVTs than its proliferative counterpart and the extent of apoptosis is associated with augmented Fas and Fas ligand expression and reduced Bcl-2 protein expression (1). The controlled invasion of EVTs into the decidua is an essential process for early placental development and the maintenance of early pregnancy. Nevertheless, the molecular mechanism involved in EVT invasion to the decidua has been poorly understood.
In this paper, the vital roles of progesterone (P4) and 3, 5, 3'-triiodothyronine (T3) in the regulation of invasive potential of EVTs in early placental development are summarized on the basis of our recent studies.

Progesterone in the regulation of the invasion of EVTs

Clinically, progesterone (P4) is used in the treatment of threatened abortion, prevention of recurrent miscarriage and in the luteal support in assisted reproduction program. However, little is known about the molecular mechanism of P4 in the regulation of EVTs function. Thus, in order to evaluate the effects of P4 on apoptosis in EVTs, we first examined the presence of progesterone receptor (PR) protein in the human trophoblast-derived HTR-8/SV neo cells, which is a possible model of human EVTs (2), and then determined the effects of P4 on the expressions of Fas, Fas-L, Bcl-2, caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP) in those cells.
The HTR-8/SV neo cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. When the cell population reached 50% confluence, the cells were stepped down to serum-free conditions in the presence or absence of graded concentrations of P4 (1, 10 and 100 ng/ml) for 48 h.
Immunocytochemistry and western blot analyses revealed that PR-A and PR-B are present in HTR-8/SV neo cell line, which is a possible model of EVTs, and that treatment with P4 (10 ng /ml) inhibits apoptosis in the EVT cells by down-regulating Fas, Fas-ligand, caspase-3 and PARP expression as well as up-regulating Bcl-2 expression in those cells (2). It seems that P4 may promote the invasion of EVTs to the decidua by inhibiting apoptosis of EVTs. This may explain the mechanism of the usefulness of P4 in the treatment on threatened abortion.

Thyroid hormone in the regulation of the invasion of EVTs

In clinical practice, maternal thyroid hormone deficiency has been implicated in early pregnancy loss, in-

dicating that thyroid hormone is vital for the maintenance of early pregnancy. It is known that T3 plays a crucial role in the maintenance early pregnancy through the induction of endocrine function in villous trophoblasts. The effects of T3 on EVTs, however, remain to be elucidated.

To investigate the possible role of T3 in the regulation of EVT invasion to the decidua, we have examined whether T3 affects EVT invasive potential and the expression of matrix metalloproteinase-2 (MMP-2), MMP-3, tissue inhibitor metalloproteinase-1, fetal fibronectin (FN), and integrin $\alpha_5\beta_1$ in cultured early placental EVTs. Isolation and purification of trophoblasts differentiating into EVTs were performed by the enzymatic digestion of the anchoring chorionic villi, with the use of human FN-precoated culture dishes and FN-precoated Matrigel Transwells. The cells attached to the dishes were subcultured in DMEM supplemented with 10% fetal bovine serum for 48 h and were characterized by immunocytochemical analysis for specific EVT markers. Thereafter, the cultured cells were stepped down to a 4% fetal bovine serum condition and cultured in the presence or absence of graded concentrations of T3 (10^{-7} to 10^{-9}M) for the subsequent 72 h.

Using FN-coated dishes and FN as a molecular glue of early placental EVTs, we successfully established a primary invasive EVT culture system. The cells attached to the FN-coated dishes were immuno-positive for cytokeratin 7, hPL and ErbB2, but immuno-negative for ErbB2, confirming that the cells are EVTs. RT-PCR and immunocytochemical analysis confirmed that T3 receptor mRNA (a 212-bp c-erb A,1 transcript) and protein are present in early placental EVTs (3).

In cultured early placental EVTs, treatment with T3 (10^{-8} M) reduced the expression of Fas and Fas-ligand as well as the cleavage of caspase-3 and PARP and suppressed apoptosis in those cells (3). Furthermore, Matrigel invasion assay revealed that T3 treatment remarkably increased the number of cell projections of EVTs over the membrane of Matrigel. Consistently, T3 treatment increased the expression of MMP-2, -3, fibronectin (FN) and integrin $\alpha_5\beta_1$ mRNA in the cultured EVTs (4). These findings suggest that T3 promotes the invasion of EVTs to the decidua by suppressing apoptosis and by up-regulating the expression of MMPs and integrin in early placental EVTs. This may explain the mechanism of thyroid hormone for the vital role in maintaining early pregnancy (5).

Conclusion

In conclusion, we have provided evidence that PR and thyroid hormone receptor exist in early placental EVTs and that both P4 and T3 promote EVT invasion to the decidua by suppressing apoptosis of EVTs and by.augmenting the expression of integrins and MMPs in those cells. This may explain, at least in part, the crucial role of an adequate supply of progesterone and thyroid hormone in the maintenance of early pregnancy and suggest the implication of maternal progesterone and thyroid hormone insufficiency in early pregnancy loss.

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Are estrogen metabolites carcinogenic?

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Introduction

The main fear of the women treated with estrogens remains breast cancer. For this reason main focus of research on hormone therapy is put on this disease, regarding clinical studies as well as experimental research, which also is included in several extensive collaboration projects between the university women's hospital of Tuebingen, Germany, and university women's hospitals of Beijing and Shanghai, China. First results of our research on the importance of the choice of progestogens for the risk of breast cancer using HRT we recently published elsewhere (1-5). In the present review we summarize particularly our view, including own research, on the importance of estrogen metabolites on possible carcinogenesis.
The World Health Organization (WHO) has classified estrogens and estrogen/progestogen combinations as 'carcinogenic' (6) although this statement has not been accepted from societies like the International Menopause Society and International Society of Gynecological Endocrinology (7,8). Regarding mechanisms for a possible increase of breast cancer risk, two main pathways are discussed: 1) increased proliferation and/or 2) development of carcinogenic estrogen metabolites.
Experimental research never can replace clinical studies, however, can evaluate the mechanisms of actions. Results of clinical studies so far suggest that estrogens indeed could be "carcinogenic", but also "carcinoprotective action" should not be excluded, which is obvious derived from the only placebo-controlled clinical endpoint study performed on this issue.

Carcinogenesis with estrogens in view of clinical studies

The Women's Health Initiative (WHI) is the only available double-blind placebo-controlled study on the issue of breast cancer risk using HRT, and demonstrated, that this risk during combined estrogen/progestin therapy is real if women are treated more than five years (Hazarad Ratio, HR 1.24; 95% CI 1.01-1.54) (9). In contrast in the estrogen-only arm of WHI no increase but rather a reduction of breast cancer risk was evaluated (10) In women compliant to estrogen treatment there was even a more than 30% significant reduction in the risk of breast cancer (HR 0.67: 95% CI 0.47-0.97) (11). This result indicates a negative effect of progestogens concerning the breast cancer risk, whereby this risk might be dependent on progestogen type and dosis, which is one of the main topics of the research on breast cancer risk and hormones (4).
But also with estrogen-only treatment an *increased* risk should not be excluded. At least 20 observational studies with estrogen-only therapy or with not well-defined regular progestogen addition (as often usual in the earlier years) have demonstrated an increased risk, as published by the Oxford Collaborative Group within a large reanalyses of 51 studies (12), although the results of these studies until today are under controversial debate (13). For example the latest evaluation of the Nurses Health Study, the most famous observational study with HRT, demonstrated no risk during 15 years of treatment but thereafter a significant increase for breast cancers although the patient sample of this subgroup with long estrogen-only treatment was very small (14). However, derived from clinical stud-

ies, it seems very clear that the main risk of breast cancer must be attributed to the progestogen component of HRT.

Carcinoprotection with estrogens – can this be plausibel?

The primarily unexpected protection from breast cancer by using estrogen-only therapy recently has been confirmed by further evaluations of the WHI data (15). Discussing this result, the WHI investigators do not argue for a statistical effect by chance but are discussing plausible mechanisms for this, like proaptotic estrogen effects especially after longer withdrawal of endogenous estrogen production ('gap hypothesis') (11,15,16). Another explanation might be, that estrogens can be metabolized to carcinoprotective substances like 2-methoxy-estradiol, as discussed below.
Different results in clinical studies using estrogen-only in postmenopausal women (showing increase, but also decrease of breast cancer risk) may be explained that the netto effect evaluated in statistical analyses is dependent on the population tested in the study: if more women are able to inhibit proliferation of preexisting cancer cells before the cancer is diagnosed clinically, the statistical evaluation will calculate a decrease of risk, if more women are not, the result will be an increase.

Carcinogenicity needs increased proliferation

The question for a *causal* relationship to breast cancer involves the production of *new* cancer cells followed by increased and long proliferation up to clinical detectable breast cancer. Own research suggest that the proliferating effect of estrogens (without addition of progestogens) is low compared to the effects of stromal growth factors (17). With estrogens-only there is no proof of inducing new cancer cells due to increased proliferation followed by errors in reduplication and mutation but this might be possible together with apocrine effects induced by strong-proliferating stromal factors like insulin growth factor, epithelial growth factor etc (17). Although by cooperative effects between estrogens and stromal factors the DNA repair can be hampered due to strongly increased proliferation rate, a whole battery of mechanisms can work to protect from reduplication errors (17,18).
Regarding carcinogenesis by stimulating already preexisting malignant breast cell proliferation, the additon of progestogens is most important, because certain progestogens can increase the cell proliferation rate which could lead in shorter time to clinical cancer as discussed on the basis of recent own investigations elsewhere (4,5).

Main pathways of estradiol metabolism

The nomenclature of hydroxy-estrogen metabolites and enzymes of of the cytochrome P450 family involved in this metabolism is shown in Figure 1. The main primary metabolites formed by A-ring metabolism are 2-hydroxy-estrone and 4-hydroxy-estrone, and by D-ring metabolism 16-alpha-hydroxy-estrone and estriol (Fig. 2a). Most primary estrogen metabolites undergo an additional degradation step by conjugation, either by glucuronidation, sulfation, or methylation (19).
Estradiol metabolites are not, as was previously assumed, inactive metabolic products destined for excretion, but may have important functions to fulfil. One such function seems to be the maintenance of homeostasis in the cardiovascular system, as demonstrated in several studies and also work of our own (20). In the context of the discussion on the "carcinogenic" property of estrogens it is becoming more and more evident that certain estradiol metabolites produced in secondary metabolic reactions also can influence carcinogenesis.
Currently research focuses on the possible carcinogenic estrogen metabolites which can be produced by secondary metabolism as shown in Figure 2b, where especially the 4-hydroxy-estrogens and 16-alpha-hydroxy-estrone may be precursors for toxic quinones (21). On the other hand there is a potent anticancerogenic estradiol metabolite, i.e. 2-methoxy-estradiol. This metabolite may counteract possible procarcinogenic activities of other estradiol metabolites and the individual metabolite pattern might be decisive for carcinogenesis.

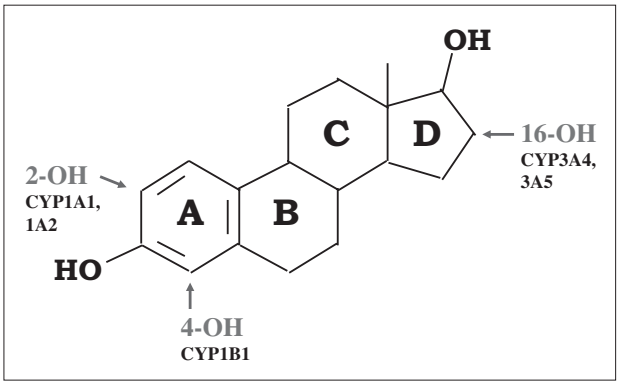

Fig. 1 - Nomenclature of steroid ring, 17ß-estradiol-hydroxy-metabolites and main metabolizing enzyme of cytochrome P450 family.

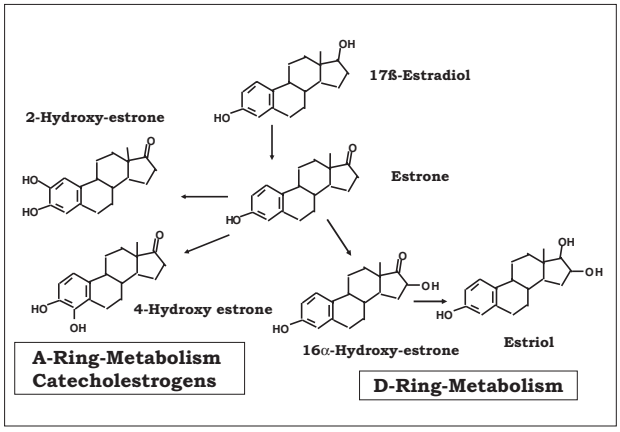

Fig. 2a - Primary A-ring and D-ring estradiol metabolism.

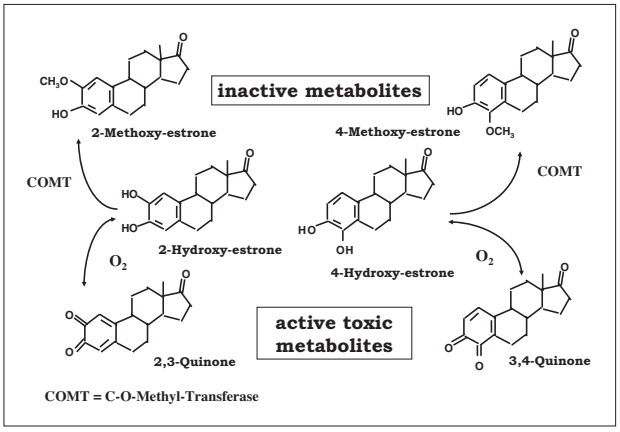

Fig. 2b - Secondary estradiol metabolism producing active toxic metabolites.

Table 1 summarizes the most important estradiol metabolites and their main biological activities derived from experimental research.
Newer investigations indicate that local estradiol metabolism particularly may have a high biological significance (22). Measurements in tissues, blood or urine may reflect such local changes, and are subject to intensive research which can be conducted only in a few specialised groups due to the highly sophisticated laboratory methods needed. The measurement of metabolites may achieve more significance in the near future, due to the new possibility to measure even small concentration changes in the systemic circulation.

4-Hydroxy-estrogens

The 4-catechol-estrogens can stimulate growth of the human breast cancer cell line MCF-7; the effect of 4-hydroxy-estradiol is stronger than that of 4-hydroxy-estrone (23). In tumour models in hamsters, rats, and mice, the 4-hydroxy-estrogens have carcinogenic effects leading to kidney tumours in hamsters, and to liver tumours in rats and mice (24). They are relatively unstable, i.e. they can be transformed into highly reactive quinones (25). Adducts of DNA with 4-hydroxy-estrogen-quinones are unstable DNA compounds, which lead via depurination to destruction of the DNA including oxidative processes, leading to DNA strang breaks and to mutations.
Elevated 4-hydroxylase enzyme activity has also been found in human breast cancer specimens (26). 4-hydroxy-estradiol was found in high concentrations in human breast cancer tissues (27,28). in addition, concentrations of quinones were significantly higher in the cancer tissue as compared to control tissue (28). Animal experiments showed a mutagenic effect of 4-hydroxy-estradiol-quinones (29,30).

Importance of "2/16 ratio of Hydroxy-estrogens" for carcinogenesis?

Observational trials have demonstrated that the ratio of 2- to 16-alpha-hydroxy-estrone (2-OHE1/16-OHE1) is decreased in women with breast cancer. However, existing studies have had different designs, have been conducted in different populations, and some have extremely small sample sizes. Thus their results are inconsistent, with some showing a strong inverse association of increasing levels of the 2-OHE1/16-OHE1 ratio with breast cancer (31,32), some showing a mod-

TABLE 1 - MAIN PROPERTIES OF BIOLOGICAL ACTIVE ESTRADIOL METABOLITES.

4-Hydroxyestrone, 4-Hydroxyestradiol
- carcinogenic in animal models
- formation of quinones with a long half-time
- proliferative in human mammary tumor cells
- slow inactivation by methylation (COMT)

2-Hydroxyestrone, 2-Hydroxyestradiol
- not carcinogenic in animal models
- formation of quinones with a short half-time
- antiproliferative in human mammary tumor cells
- rapid inactivation by methylation (COMT)

2-Methoxyestradiol
- strong antiproliferative effect on various tumor cells
- potent inhibition of neoangiogenesis high potency to capture radicals

16alpha-Hydroxyestrone
- increase of incidence of mammary tumors in the mouse
- mutagenic effect on target cells by DNA-adducts
- proliferative effect on tumor cells
- higher concentrations in tumor tissue of the breast
- metabolism to estriol or end product

2-Hydroxyestron : 16a-Hydroxyestron
Decrease: DMBA, pesticides, nutrition (high fat, low fibers), neoplasia
Increase: sports, nutrition (low fat, high fibers), isoflavones, indole-3-carbinol

est association (33-36), and still others showing no association (37).
A study conducted in China (34) found an inverse association of the 2/16 ratio with breast cancer using urine samples collected before surgery but a positive association using postsurgery samples. In an own case/control study including 144 women with breast cancer and 292 controls, respectively, we found lower excretion of 2-hydroxy-estrone and higher excretion of 16-alpha-hydroxy-estrone in the cases as compared to controls, but only in postmenopausal women (38).

Carcinoprotective 2-Methoxy-estradiol

The conversion of 2-hydroxy-estradiol by the enzyme catechol ortho-methyltransferase (COMT) leads to 2-Methoxy-estradiol (2-ME). The growth of breast cancer cells (and other tumour cells) has been inhibited by 2-ME (39). A finding of major significance for tumor research was the detection of an antiangiogenic action of 2-ME and its strong potency to capture radicals like semiqinone radicals produced during secondary estrogen metabolism, which may be involved in carcinogenesis of breast cancer (40,41).
We found that the combination of 2-ME with tamoxifen elicits additive antiproliferative actions in human breast cancer cells (42). 2-ME was also able to increase the effect of certain cytostatics in breast cancer cells (43). In addition we found a similar inhibition of the aromatase enzyme by 2-ME as compared to letrozole (42). Thus 2-ME is a potent antiproliferative metabolite decreasing proliferating activity of the mother substance estradiol.
Thus during metabolism of estradiol not only potential genotoxic, but also carcinoprotective metabolites can be produced which may be one of the explanation of the result of WHI during treatment with estrogens-only (10,11,15). In general regarding regulations in human endocrinology, there are many examples that the organism can regulate in two contrary directions, in this case stimulation of cell proliferation (estradiol) as well as inhibiting negative effects (estradiol metabolite 2-ME). Currently the use of 2-ME is tested in patients with refractory metastatic breast cancer.

Factors influencing estradiol metabolism

The assessment of estradiol metabolism has to consider factors which can influence this metabolism. For example endocrine diseases such as hypothyroidism or drugs like L-thyroxine and H2-antagonists can change estradiol metabolism (19). In addition, diet and physical activity may be of importance (44,45).
Our own interest has been focused on estradiol metabolism in smokers, who may produce a higher amount of potential toxic estrogen metabolites due to increased hepatic activity, as delineated elsewhere (46). Oral estrogen therapy in postmenopausal women can lead to an increase of 4-catechol estrogens as compared to non-smokers which may lead to an increased breast cancer risk (47). Using transdermal estradiol this mechanism can be avoided, however not the mechanism of proliferating preexisting cancer cells.
We also were interested if different forms of HRT regarding type of hormones and application form which may influence the pattern of estrogen metabolism (48). During treatment of postmenopausal women with different regimens of HRT we found that the estradiol metabolism can be influenced by administration route, and possibly also by the type of progestogen addition. Transdermal application of hormones in contrast to oral treatment may avoid the development of high amounts of precursors which can be further metabolized to the potential genotoxic quinones and semiquinones, due to low dosing and avoiding of the first hepatic passage (48,49,50) (Fig. 3) shows this difference between oral and transdermal estradiol treatment.
However, there is epidemiological evidence, which seems to support an increase of risk also with transdermal estrogen-only therapy: In a Finnish study an increased risk also with transdermal estrogen was observed although the subgroup was small (51). These results suggest that, although the production of estradiol metabolites may be minimized during transdermal estrogen application, still enough local estrogen concentrations may be present to promote breast carcinogenesis.
The toxic effects of 4-hydroxy-estrogens can probably be prevented under normal conditions by various cellular defence mechanisms. For example intracellularly formed catechol estrogens are rapidly methylated by by the enzyme catechol ortho-methyltransferase

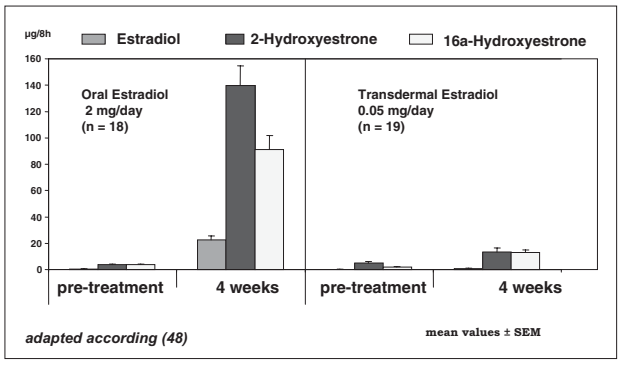

Fig. 3 - Estradiol metabolism during oral versus transdermal estradiol replacement in postmenopausal women (48).

(COMT). However, patients with a COMT defect due to genetic polymorphisms could be especially on risk of breast cancer during HRT, and similarly other polymorphisms of key enzymes in estrogen metabolites are discussed to increase the breast cancer risk, especially if there are cumulated defects. However, the clinical relevance still is under discussion, since those genetic changes are very rare (52,53).

Additive oxidative cell stress necessary for carcinogenicity

From the more detailed biochemical view the potential genotoxic effect of estrogen metabolites are not caused by the quinones (Fig. 2b) but by further intermediate metabolites (25,26,54,55). The quinones can react to "semiquinones", which, however, needs "oxidative cell stress" by a so called "one-electrone-oxidation" to form an electropositive radical structure. Cells are extremely good protected against this reaction by various defense mechanisms, e.g. 2-ME produced at the same time during estradiol metabolism can "capture" this radical substances.

Only if all protection mechanisms fail, those very reactive intermediate products are produced, and can in balance with quinones form a complex molecule ("ROS" Reactive Oxidative Substance), stable enough for further reactions with the DNA, leading to strang breaks which by oxidative DNA damage destroys the DNA structure necessary for replication. Further reactions like mutations finally can produce the first malignant cell which, however, needs proliferation up to 10-9 cells to be detected as clinically cancer e.g. by mammography.

Derived from doubling times of the most malignant breast cancer cells during estrogen treatment this carcinogenesis needs at least 10-15 years, and during this long time all the carcinoprotective mechanisms can work (17). However, if the proliferation rate is strongly increased as by addition of certain progestogens, the protection may fail and the clinically cancer can be developed.

Conclusion

Thus, to answer the question asked for this review, estrogen metabolites *per se* are *not* carcinogenic, because additional factors are needed for carcinogenesis, and the main problem using HRT is the increase of proliferation rate, e.g. by adding certain progestogens. With estrogen-only the proliferation rate is low, and various defense mechanism can operate in the physiological human body. This can prevent the formation of possible genotoxic products of estradiol metabolism which without protection would be produced during oxidative stress by so called 'one-electrone-oxidation'. Only if protective mechanisms fail, e.g. due to genetic polymorphisms of key enzymes like COMT necessary for protection, and only if the proliferation rate is strongly increased, e.g. by addition of certain progestogens, women may be at risk. Thus, only under rare special conditions it is conceivable that the human body cannot react sufficiently.

Besides proliferating effects or production of potential genotoxic metabolites, estrogen also can induce carcinoprotection like the production of carcinoprotective metabolites and apoptotic effects, which can explain even the decrease of breast cancer risk in the WHI study. The different statistical results in clinical studies may depend on the different carcinoprotective potency of women in the study. Because the excessive risk in absolute numbers related to the whole population is low (about 0.01%/year), only few women are decisive for the statistical result in studies. However, these few women may be of high risk caused by genotoxic estrogen metabolites. In the presence of factors, which negatively could influence the estradiol metabolism such as factors like genetic polymorphisms of protective key enzymes or excessive oxidative cell stress like in smokers, it seems prudent to prefer transdermal therapy to minimize the production of possible toxic metabolites.

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Conflict of interest

The authors do not have any conflict of interest to declare in context with this publication.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Breast cancer and progestogens: could certain cell components predict the risk?

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Introduction

The role of progestogen addition to estrogen therapy in the postmenopause has come into scrutiny since the results of the estrogen-only arm of the study Women' Health Initiative (WHI) are published as compared to the WHI combined arm (1,2). In contrast to the WHI combined arm, in the estrogen-only arm no increase but rather a reduction of breast cancer risk was evaluated. This result indicates a negative effect of progestogens concerning the breast cancer risk. However, the question remains still open, in as far the combination of estrogens with synthetic progestogens as well as with natural progesterone may elicit the same increased risk and/or if there might be important differences between the progestogens used in hormone therapy and hormonal contraception. In the present paper own experimental data focusing on the importance of special cell components which may be crucial for different progestogen action are summarized.

Rationale and limitations of experimental research

Despite their widespread use, *in vitro* models using cell cultures have certain limitations: the choice of culture conditions can unintentionally affect the experimental outcome, and cultured cells are adapted to grow *in vitro*; the changes which have allowed this ability may not occur *in vivo*. Limitations of *in vitro* study might be the high concentrations needed for an effective antiproliferative effect. Higher concentrations may be required *in vitro* in short-time tests in which the reaction threshold can only be achieved with supraphysiological dosages. However, higher concentrations may also be reached *in vivo* in the vessel wall or organs compared to the concentrations usually measured in the blood.
Thus in vitro experiments, although only conducted for a short time and with high pharmacological concentrations, can simulate special in vivo conditions. But comparisons on the effect of different substances (like different progestogens) should always be done in the same model, since cell culture conditions can have a strong influence on the results.
There are numerous experimental data available on the effect of progestogens on the proliferation of normal and cancerous breast epithelial cells (3-6). However, only few experiments have been done with a higher number of progestogens in the same cell model. Our working group of the University of Tuebingen, Germany, recently also in close collaboration with Chinese working groups of the University of Beijing, China, is testing systematically all available progestogens in different experimental models which have been demonstrated to be important models regarding the research of mechanisms of hormonal action in the breast. Therefore we will focus here on own experiments, in which we have compared all available progestogens in the same cell model.

Overview on own experimental research

We investigated effects on proliferation as well as on apoptosis using different cell cultures and different conditions. We tested the effect on malignant breast cells, but also on benign cells, which can be important in terms of the question, if new cancer might be devel-

oped (for example due to very fast proliferation followed by mistakes of DNA replication, leading to mutation) or if hormones only proliferate already preexisting cancer cells. In addition, a possible paracrine influence of the stroma was considered by including the most important stromal growth factors in our experiments (7-13). To our knowledge this probably important stromal influence was not incorporated in in vitro experiments so far.

In our recent experiments we also tested the importance of certain cell components which can influence the effect of hormones in terms of proliferation mechanisms and can explain differences between hormones like the impact of various progestogens added to estrogen-induced proliferation. In addition to variety of already published original papers on our experimental research regarding this issue an extensive overview of our in vitro investigations with human benign and malignant epithelial breast cells recently has been published (14). In the present summary we concentrate on experiments with human cell cultures although also animal studies can indicate differences of hormonal action, like recently also observed in own research (15), but to our knowledge up to date there is a lack of head to head comparison of the available progestogens in the same animal model.

As a summary of our earlier experiments (7-13) we can suggest that the differences shown in those experiments can lead to the conclusion that the paracrine impact of certain strong growth factors is important for the influence of some, but not of all progestogens. Even if there should be a variety of other factors regarding the rate of proliferation it could be concluded already from our earlier experiments that there are differences between the progestogens, and the choice of progestogen may be important for the risk of breast cancer, because we see large differences when tested head to head in the same model.

In vitro experiments clearly cannot replace clinical studies, but they are very useful to explore possible differences between substances, and experimental research particularly can evaluate mechanisms and demonstrate biological plausibility (16). It has to be noted that derived from clinical studies until today the risk of breast cancer during hormone therapy is not clear. For example a recent new evaluation of the "Oxford Collaborative reanalysis" of 51 observational studies (17) or even new evaluations of the data derived from WHI (18,19) using systematically basic parameters of epidemiology like the "Bradford Hill criteria" could not establish an increase of breast cancer using estrogen-only therapy, although a small increase of risk adding certain progestogens to estrogen therapy cannot be excluded.

However, in most clinical studies only medroxyprogesterone acetate or norethisterone have been tested. To our knowledge the E3N-study is the only published study in which a variety of very different synthetic progestogens have been tested in a larger cohort for longer duration (on avarage 7-8 years), all leading to significantly increased risk when combined with estrogen in contrast to progesterone and dydrogesterone (20). However, at this time the conclusion should not be drawn that it may be totally safe to treat patients with more "natural" progestogens like dydrogesterone, the retroisomer of natural progesterone. Follow up evaluations of the E3N-Study suggest that longer duration of therapy may also lead to increased risk using dydrogesterone longer than 5 years (21). Thus to evaluate a possible risk we still need the biological plausibility, i.e. experimental research, to find the best option which may reduce the risk of breast cancer using hormone therapy.

Importance of PGRMC1 for different progestogen action

Recent experimental data revealed the existence of a membrane-located progesterone receptor, called progesterone receptor membrane component 1 (PGRMC1) (10). PGRMC1 has been detected in several cancers and cancer cell lines. It is over expressed in lung cancer and colon cancer (22). We have demonstrated that PGRMC1 is present in human breast cancer tissues (23), Scientific Prize during the World Congress of Menopause, Madrid, May 2008). Recently we published first experiments comparing MPA with progesterone (24). The journal Menopause (Journal of the North American Menopause Society) published a special EDITORIAL on our original paper with a title which can express the importance of our recent experiments, because we could demonstrate strong proliferating of MPA in contrast to natural progesterone which has no effect on estradiol-induced proliferation of human breast cancer cells (25):

"Can the increase in breast cancer observed in the estrogen plus progestogen arm of the Women's Health Initiative trial be explained by progesterone receptor membrane component 1?" As conclusion we published *that "overexpression of PGRMC1 sensitizes the proliferative response of human breast cancer cells to estradiol, and the effect of progestogen on breast cancer tumorigenesis may depend on the specific progestogen used for hormone therapy and hormonal contraception".*

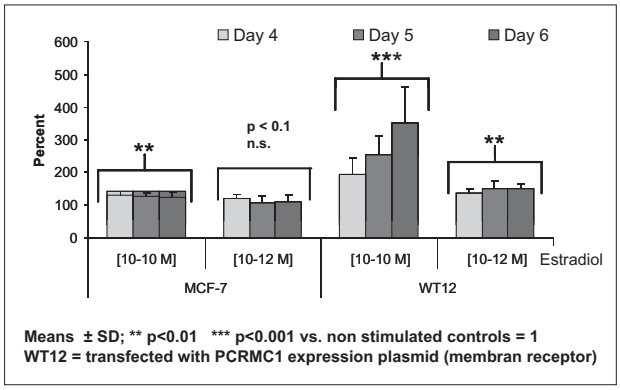
In further experiments we have investigated receptor membrane-initiated actions of various other synthetic progestogens in comparison to progesterone in human breast cancer cells (12) (Scientific Prizes during the

German Congress of Breast Oncology, Dresden, June 2011 and Christian Lauritzen Prize during the German Congress of Menopause, Frankfurt, November 2011). In these experiments MCF-7 cells were stably transfected with PGRMC1 expression plasmid (MCF-7/PGRMC1-3HA, WT-12 cells). We tested the effect of different estrogen concentrations which should suggest, if there is a dose-related dependency of breast cancer risk.

Figure 1 shows our recent results demonstrating the importance of PGRMC1 for estrogen induced proliferation which is clearly dependent on the estradiol-concentration which was much more pronounced in WT-12 cells (26). This effect could be completely abrogated by the addition of the E2-antagonist fulvestrant.

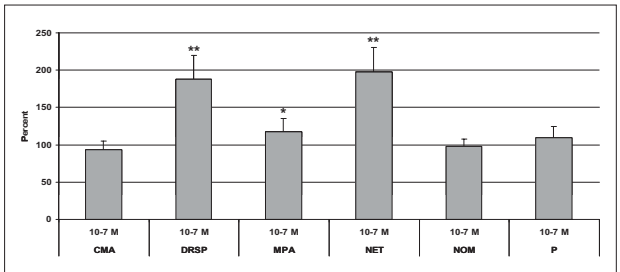
Further we tested all availabe synthetic progestogens in comparison with natural progesterone, i.e. head to head comparison in the same model, which should suggest if their might be principal differences between different progestogens used in hormone therapy and in hormonal contraception (27,28). For these new experiments we tested the effects of progesterone (P) and the synthetic progestogens chlormadinone acetate (CMA), desogestrel (DSG), dienogest (DNG), drospirenone (DRSP), dydrogesterone (DYD), levonorgestrel (LNG), medroxyprogesterone acetate (MPA), nomegestrol (NOM) and norethisterone (NET) on cell proliferation. MCF-7 and MCF-7/PGRMC1-3HA (WT-12) cells were stimulated with different concentrations (10-9 M to 10-7 M). In MCF-7 cells DNG, DSG, DYD, LNG and NET increased the proliferation at 10-7 M, the effect being highest for NET with about 20%. In WT-12 cells the same progestogens, but additionally DRSP and MPA showed a significant increase, which was much higher (30-245%) than in MCF-7 cells. Here again NET showed the highest proliferative effect. No effect was found for CMA, NOM and P.

Figure 2 shows the effects comparing 6 of the 10 tested progestogens at the same concentration (10-7 M); Fig. 3 shows the results for the 4 progestogens which at this time are mostly used in hormone therapy, i.e. MPA, NET, DRSP in comparison with natural progesterone. In the experiments summarized in Fig. 4 we tested the difference between sequential and continuous combined estrogen/progestogen combinations (at two different concentrations) which are the both most used regimens in hormone replacement therapy. Estradiol (10^{-10} or 10^{-12} M) was sequentially or continuously combined with progesterone or with different synthetic progestogens. Cell proliferation was investigated in MCF-7 and MCF-7/PGRMC1-3HA (WT-12) cells. In both sequential and continuous combination E2 alone elicited a significant 2-3 fold proliferative increase at 10^{-10} M. This increase was not significantly influenced by the addition of the various progestogens. However, E2 alone at the lower concentration of 10^{-12}M showed no significant effect on the proliferation of WT-12 cells, whereby addition of



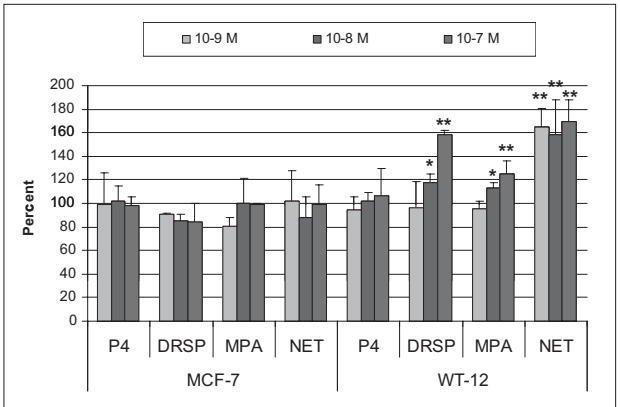
Means ± SD; ** p<0.01 *** p<0.001 vs. non stimulated controls = 1
WT12 = transfected with PCRMC1 expression plasmid (membran receptor)

Fig. 1 - Proliferation of breast cancer cells: dependency on estrogen dosage and treatment duration.



Means ±SD; * p<0.05; ** p<0.01 vs. controls = 100%
MCF7-WT12cells = transfected with PCRMC1 expression plasmid (membran receptor)
CMA Chlormadinone acetate, DRSP Drospirenone, MPA Medroxyprogesterone acetate, NET Norethisterone, NOM Nomegestrol, P Progesterone

Fig. 2 - Proliferation of breast cancer cells: dependency on progestogen type.



Means ±SD; * p<0.05; ** p<0.01 vs. controls = 100%
WT12 = transfected with PCRMC1 expression plasmid (membran receptor)
P4 Progesterone, DRSP Drospirenone, MPA Medroxyprogesterone ac., NET Norethisterone

Fig. 3 - Proliferation of breast cancer cells: dependency on progestin dosage and progestin type.

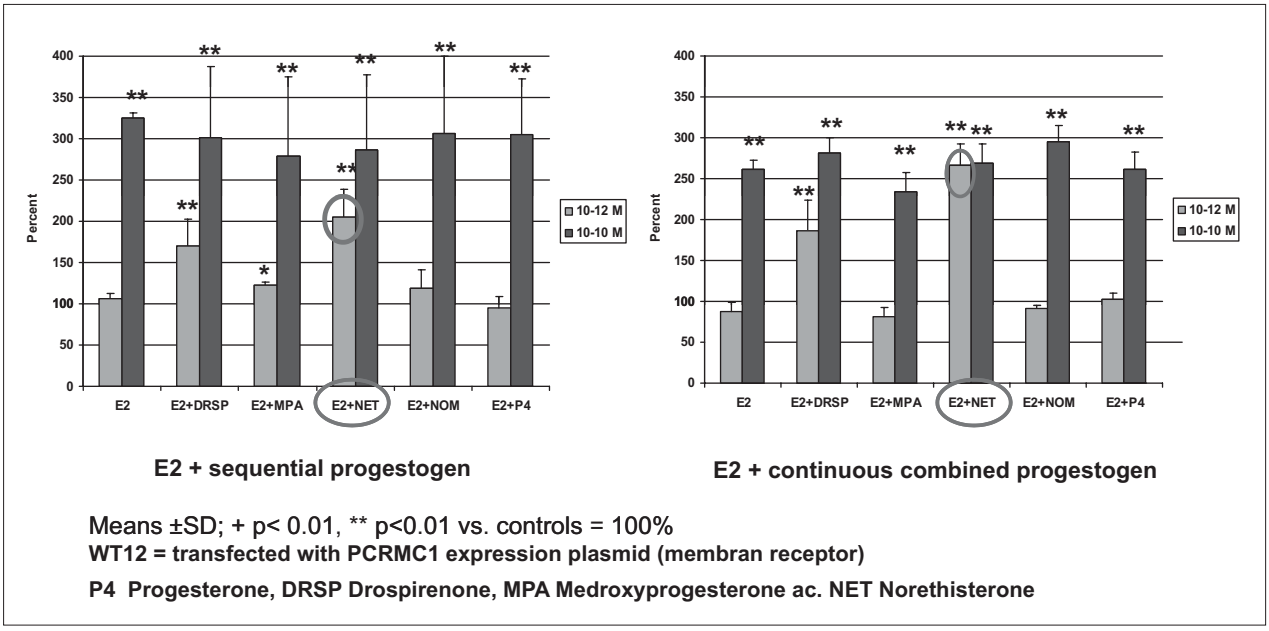


Fig. 4 - Proliferation of breast cancer cells: dependency on estrogen/progestogen regimen (used in two different concentrations).

DRSP, MPA and NET triggered a significant proliferative response, the increase being higher for the continuous combination, especially combining NET with E2. In contrast NOM and P were neutral in this action, i.e. elicited no effect.

Our present conclusion of all the experiments in malignant cells expressing PGRMC1 compared to other malignant cells is, that certain synthetic progestogens can trigger a proliferative response of PGRMC1 overexpressed human breast cancer cells, and that the effect of progestogens on breast cancer tumorigenesis may clearly depend on the specific pharmacology of the various synthetic progestogens.

In the present research we try to find a possibility for routine screening of women overexpressing this obviously important cell component which could lead to a decision if women can be treated or not using certain progestogens in hormone therapy.

Importance of RANKL for different progestogen action

Also other special cell components may be important for increased proliferating action of certain progestogens.

In vivo administration of MPA can increase the risk of breast cancer in mice by activating the protein RANKL (receptor activator of NF-Kappa B ligand), known as the key osteoclast differentiation factor) in normal and premalignant in mammary-gland epithelial cells. (29,30). Genetic inactivation of the RANKL receptor RANK in mammary-gland epithelial cells prevents MPA-induced epithelial proliferation, impairs expansion of the CD49fhi stem-cell-enriched population, and sensitizes these cells to DNA-damage-induced cell death. Deletion of RANK from the mammary epithelium results in a markedly decreased incidence and delayed onset of MPA-driven mammary cancer. Until now it is not known if mechanisms working via this RANKL/RANK system would be different using different progestogens, and the question remains if this by the end could lead to cancer clinically observed during treatment with hormone therapy.

Further research should suggest if screening regarding this cell components might be an option to assess the women who might be of increased risk during treatment with certain regimens of hormone therapy, especially with respect to the choice of the progestogen component.

It should be mentioned that those questions also might be important not only for hormone therapy of postmenopausal women but also in the field of hormonal contraception because the same or similar progestogens are used. However, we already have been able to show that the primary estrogen-induced proliferation using ethinyl estradiol compared to estradiol is much lower in rate and degree (31,32). Obviously the breast is an important endorgan for the natural estradiol but not for the synthetic estrogen, i.e. ethinyl estradiol, which main effects are in the liver. Thus the additional proliferating effects like with growth factors

or by addition of the progestogen to estrogen induced proliferation might be not such important. Presently this question is under our research.

Conclusion

In earlier experiments we and others were able to show that the paracrine effects of strong proliferating stromal factors have to be considered which might be modulated by certain progestogens leading to faster breast cancer cell proliferation. In addition recent research suggest that not only the classic genomic mechanisms are working but there is a cross-talk with certain cell components, especially located in the cell membrane of epithelial cancer cells, which can enhance the proliferating effect of certain synthetic progestogens like MPA or norethisterone, if added to estrogen, in contrast to natural progesterone. Thus experimental data with the comparison of various progestogens in the same models present a rather high evidence that there may be differences between the various synthetic progestogens regarding the breast cancer risk during hormone therapy and that the use of natural progesterone may be an important option to reduce this risk.

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Conflict of interest
The authors do not have any conflict of interest to declare in context with this publication.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Progesterone receptors and pregnancy

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Introduction

The steroid hormone progesterone plays a central role in the reproductive events associated with pregnancy establishment and maintenance as well as delivery (1). Physiological effects of progesterone are mediated by the action of the hormone with specific intracellular progesterone receptors (PRs) (2).
Progesterone action is mediated via progesterone receptors (PRs) using genomic and non-genomic pathways (3). This presentation is exclusively concerned with the genomic action of progesterone in pregnancy. There is one progesterone receptor gene present, which leads – estrogen-dependent – to different progesterone receptor isoforms called progesterone receptor A (PRA) and progesterone receptor B (PRB) and possibly other isoforms such as progesterone receptor C (PRC). PRA is shorter by 164 amino acid at the amino acid terminal end of the receptor. PRA appears necessary to allow the progesterone dependent physiological responses in the female reproductive tract. PRA is required for normal ovarian and uterine function, but is dispensable in the mammary gland. While PRB appears to be required for maintaining quiescence of the uterus but also appears to be required for instance that progesterone exerts its proliferatory effects on the breast. PRB is the principal mediator of progesterone action (4). PRA represses the transcriptional activity of PRB (5). The extent to which PRA represses PRB mediated transcriptional activity is directly related to its abundance relative to PRB (PRA to PRB ratio) (6).
Selective ablation of PRA and PRB in mice allowed to look for the contribution of the individual PR isoforms to the pleiotropic reproductive activities of progesterone. In PRA knockout mice, in which the expression of the PRA isoform is selectively ablated results in severe abnormalities in ovarian and uterine function. In PRB knockout mice it was shown that ablation of PRB has no effect on ovarian and uterine response to progesterone, but rather results in reduced mammary ductal morphogenesis. Thus PRA is both necessary and sufficient to elicit the progesterone dependent reproductive responses necessary for female fertility, while PRB is required to elicit normal proliferative responses in the mammary gland to progesterone (2). But also other progesterone receptor isoforms have been reported such as PRC. PRC is described as an amino acid terminal truncated progesterone receptor isoform and enhances progesterone induced transcriptional activity (7). PRC has been reported as a 60-kDa protein that upon hormone binding, translocates from the cytoplasma to the nucleus and modulates the transcriptional activity of PRA and PRB. However, the existence of the PRC has been questioned (8). From early to late pregnancy the various events will be scrutinized for the role of progesterone and progesterone receptors.

Implantation

Besides the proper conversion of the endometrium into a secretory state on crucial aspect appears to be decidualized endometrial stromal cells and extravillous trophoblast invasion, which is governed by progesterone action genomically via nuclear progesterone receptors (n-PRs) and membrane progesterone receptors (m-PRs). The temporal expression pattern of progesterone receptors in the uterine luminal epithelium supports their requirement during early events of implantation (9).

Blood flow

Blood flow in the corpus luteum phase is related to progesterone, which is reflected in progesterone receptor activity. The relatively high resistance index during the early follicular phase declines with progress towards the luteal phase. By the mid-luteal phase the resistance index is low, which corresponds with a high blood flow to the corpus luteum. When the corpus luteum regresses, reflected by a decrease of progesterone there is an increase in the resistance index again and therefore a reduced blood flow. When implantation occurs placental HCG stimulates progesterone synthesis and secretion by the corpus luteum and the resistance index decrease and remains low throughout a normal pregnancy. This is not the case when preeclampsia develops. The resistance index increases as an early warning sign for the possibility that a clinical manifestation of preeclampsia may occur (11,12).

Immunology of pregnancy

The activation of the immune system is necessary for normal pregnancy. This is possible through a number of events:

1. Up-regulation of PRs in natural killer cells in the decidua and in lymphocytes among placental cells (13).
2. Progesterone causes via progesterone receptor positive lymphocytes and decidual CD 56+cells a 34kDa immune regulatory protein known as progesterone-induced blocking factor (PIBF) and appears to be the pivotal mediator in progesterone dependent immune modulation (14).
   a. PIBF enhances asymmetric antibody production
   b. PIBF causes Th2-/Th1-cytokine shift
   c. PIBF inhibits natural killer cell activity (15)

The profound effect of progesterone on regulation of the immune system in pregnancy is also exemplified by the differentiation of T-cells into T-regulatory (Treg) cells on the one hand and progesterone suppresses the differentiation of CD4 T-cells into inflammation-associated Th17 cells on the other hand. So progesterone by regulation of cell differentiation promotes immune tolerance (16). Also other studies have indicated that progesterone acts as a critical regulator of Treg cells in pregnancy from CD14 + CD25+T-cells (17,18).

Progesterone-controlled T-cell cytokines for immunological regulation and maintenance of pregnancy

Progesterone is associated with a switch from a Th1- to a Th2-cytokine pattern at the maternal-fetal interface. Progesterone promotes the production of Il4 and Il5. Leukaemia inhibitory factor (LIF) –essential for embryo implantation- associated with Th2-cytokines. This is up-regulated by Il-4 and Il-5. On the other hand LIF is down-regulated by Th1-cell inducers (II-2, IFN-γ und INF-α). There is a decreased production of LIF, II-4 and II-10 by decidual T-cells in women with unexplained recurrent miscarriage.
It can be concluded that LIF and/or Th2-cytokines contribute to the maintenance of pregnancy (19). Increasing INF-γ: II-4 and TNF-α: II-4 ratios have also been found in women with recurrent failed pregnancy (20).

Actions of progesterone/progesterone receptors throughout pregnancy on the uterus

Already in the 1950's Csapo proposed the progesterone hypothesis (21). This means pregnancy preservation by actively blocking endometrial contractions. In many species it was proven that progesterone is essential in the maintenance of pregnancy and the disruption of its production or activity initiates labor. In the 1970's Csapo demonstrated contractility in relation to circulating progesterone. In humans removal of the corpus luteum before the eights week of gestation started contractility of the corpus uteri together with the widening of the cervical canal and shortening of the cervix in the first trimester leading to miscarriage. This could be avoided by progesterone treatment (22, 23). This was followed by studying progesterone receptors. Besides the classical progesterone action via nuclear progesterone receptors (nPRs A+B) there are also membrane progesterone receptors (mPRs α+β) (3, 24).
Progesterone - progesterone receptor interaction can affect cellular function either via the genomic pathway (by modulating gene expression) or non-genomic pathways such as interaction with the different membrane progesterone receptors (α, β, γ) and progesterone membrane component-1 and -2 (PGRMC 1 and PGRMC-2) and other cell surface receptors such as oxytocin receptor and GABA a-receptor linked with intracellular signalling cascades and direct activation of intracellular signal molecules (3). There are numerous theories on hormonal control of labour onset. Most consider oxitocin, prostaglandins and steroid hormones as primary regulators of the initiation of labour. For starting labour and parturition in humans a functional progesterone withdrawal seems to be responsible. During normal human pregnancy progesterone promotes via PRB myometrial relaxation. The functional progesterone withdrawal of parturition can be mediated by a decrease of progesterone receptors

rather than increased estrogen receptors (ERs) (25) or by an increased myometrial expression of PRA, which results in an increase in the myometrial PRA to PRB ratio (26).
Cortisol induces PRA in human amnion fibroblast. Induction of PRA attenuates the induction of cytosolic phosholipase A2α expression by cortisol in human amnion fibroblast leading to increased prostaglandin synthesis (27,28). Another possibility of labour initiation is up-regulation of the progesterone receptor C isoform (29).
The result of effective treatment with a progestogen like 17αhydroxyprogesterone caproate was thought to be through inhibition of the endogenous progesterone metabolism resulting in an increased concentration of progesterone at the local receptor level (30). Histologically it was shown that PRs were significantly higher in decidual cells from pre- versus post-labour contraction decidua basalis and parietalis sections (31).
Furthermore, progesterone receptor membrane component 1 (PGRMC1) is lower in myometrium of women at term either not in labour (p=0.004) or in labour (p=0.05). Compared with tissues in women in preterm non-labour, PGMRC1 levels are also decreased (p=0.02) in myometrial tissues from women during preterm labour compared with preterm non-labour. Progesterone rapidly inhibits contractions of myometrial tissue compared to control (p_0.05) in vitro. PGRMC1 mediate the non-genomic action of progesterone and the relaxation effect on human myometrium during pregnancy (32,33). However, progesterone does not inhibit stretch –induced MAPK activation or gene expression explaining most likely why progesterone is ineffective in the prevention of preterm labour in multiple pregnancies (34). Contraction associated proteins (CAPs) appear to be the link between progesterone and progesterone receptor. Recently a novel pathway in which progesterone represses the expansion of two CAP genes: Conexin 43 C (CNX43) and the oxytocin receptor gene was described (OXTR) (36,37). They also found that levels of 2miRNAs belonging to the mi-RNA-200-family increases in the mouse and human myometrium with advancing gestation and in parallel with CNX43 and OXTR. The immediate miRNA-200 targets were factors that down regulate CAP levels. They identified two repressive transcription factors ZEB1 and ZEB2. ZEB1 and ZEB2 repress the expression of CNX43 and OXTR in mouse and human endometrial cells. In addition it was found that ZEB1 and ZEB2 inhibited expression of members of the miRNA-200-family suggesting that these proteins and miRNAs form a mutually regressive negative feedback loop in myometrial cells (35,36). There appears to be a significant difference between allel and haplotype frequencies in the PR among women with preterm birth and women with preterm birth and a family history of preterm birth (37). The author suggests that progesterone receptor may be a candidate for association with preterm birth or familiar preterm birth.

The role of progesterone and progesterone receptors during the first trimester of pregnancy

In early pregnancy many studies have indicated a strong relationship between the levels of circulating progesterone and the clinical outcome of pregnancy (38). In a more recent study cytosol and nuclear PRs were measured in the endometrium of women with habitual abortion compared with fertile women and also the corresponding circulating plasma progesterone levels (39). The data are summarized in table 1. There is a highly significant difference of circulating plasma progesterone comparing women with habitual miscarriage and fertile women (p<0.005). This is also clearly shown for cytosol nuclear PRs. In a recent study it was evident that a significant low PRA expression was found in trophoblast cells of miscarriages (40).
Since extravillous trophoblast invasion is related to available progesterone, which is reflected in progesterone receptor levels as shown in table 1. Particular limited trophoblast invasion is associated with spontaneous abortion (41). Reduced extravillous trophoblast invasion results in nonproper spiral artery development (42).
These limitation of invasion may be not only responsible for early pregnancy loss and spontaneous and recurrent miscarriages but also for missed abortion, preterm labour and early or late onset of preeclampsia – a clinical spectrum of different degrees of deficient trophoblast invasion.
Investigations were undertaken to study the genpolymorphism of PR in women with spontaneous recurrent miscarriages. In Thai women it was found that PR polymorphism confers acceptability to idiopathic recurrent pregnancy loss (43). Furthermore, the clinical efficacy of progesterone treatment for prevention of

TABLE 1 - COMPARISON OF PLASMA PROGESTERONE AND PROGESTERONE RECEPTOR CONTENT OF THE ENDOMETRIUM IN FERTILE WOMEN AND IN WOMEN WITH RECURRENT MISCARRIAGE (ACCORDING TO 39).

	Progesterone ng/ml	PR cytosol fmol mg/protein	PR nuclear fmol mg/protein
Fertile women	8,8 ± 1,6	21 ± 6,8	30 ± 11,8
Habitual miscarriage	4,0 ± 1,5	<3	<3
Significance	p<0.005		

idiopathic recurrent pregnancy loss might be progesterone receptor gene polymorphism (44).
However with similar studies in Brazil and India no correlation was found in any of the investigated polymorphism and it was concluded that the studied gene polymorphisms are not the major determinant of pregnancy failure (45,46).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Clinical cardiovascular trials of HRT

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During the 1990s, hormone replacement therapy (HRT) was used to reduce heart disease risks, in addition to treating menopausal symptoms. This was based on evidence from large observational studies that HRT provided cardioprotection. However, it was not clear whether HRT increased breast cancer risk.

Cardiovascular disease (CVD) is the leading cause of death in women and increases with aging and menopause. Evidence suggests that endogenous estrogen contributes to delaying the onset of atherosclerotic CVD events in women. Studies in animal models and women provide biological plausibility for the concept that estrogens can exert atheroprotective effects via both systemic effects on circulating factors and direct effects on the heart and blood vessels (1,2). These observations allowed formulating the hypothesis that estrogen-based HRT could reduce CVD risk in postmenopausal women. Besides, HRT has proved to reestablish the deteriorated lipoprotein profile frequently observed in postmenopausal women.

In 2002, the first published results of the Women´s Health Initiative (WHI) Study provided a major source of data for this analysis (3). The WHI study was undertaken to determine, under the conditions of a randomized controlled trial, whether HRT truly protected against heart disease and whether or not it increased breast cancer risk**.** Although this study was categorized as a primary prevention trial for coronary heart disease, the mean age at recruitment was 63 years, when menopausal symptoms have usually finished and HRT is rarely started, and only 3.5% of the women were 50-54 years old, the age when women usually make a decision regarding initiation of HRT. This point was not acknowledged by the researchers but instead, they concluded that HRT was not cardioprotective and that its risk–benefit ratio did not favor the use of postmenopausal hormones for prevention of chronic diseases. As a consequence, there was an important change in prescription habits following recommendations to reserve HRT for very symptomatic women, and to limit its use to the 'shortest duration needed' and to 'the lowest effective dosage'. After publication of the WHI findings, a number of studies have examined the effects of HRT in 50- to 55-years-old women more likely to consider starting HRT.

Recently, the American Endocrine Society Scientific Statement has provided an overview about HRT use in postmenopausal women (4). Regarding the quality of evidence the Statement concluded that evidence from the WHI trial is weighted less than that of a randomized controlled trial according to the GRADE system criteria because of mitigating factors: large dropout rate; lack of adequate representation of applicable group of women (i.e. those initiating therapy at the time of menopause); and modifying influence from prior hormone use. For this reason, many of the conclusions from the WHI are judged as level B evidence (4).

Moreover, in relation to the analysis of the benefits and risks of HRT in women recently menopausal (i.e. ages 50-59 or <10 years postmenopausal), different re-analyses of the WHI indicated the important influences of age and time since initiation of HRT on benefits and risks (5,6). Because most women start HRT shortly after menopause, available data regarding these women were specifically analyzed. Results are summarized as the excess number of women experiencing benefit or risk per 1000 women using HRT for 5 years of more.

Therefore, a central issue of discussion in interpreting the findings of the WHI study is the extent to which the effects of HRT are influenced by the timing of its

initiation, in terms of either the age of the recipient or the duration of estrogen deficiency (i.e. "time since menopause") (7). This "timing hypothesis, that HRT prevents CHD when administered soon after menopause or in younger women but not if initiated later in menopause or in older women, is supported by animal data and by some human studies (8). Furthermore, the reduction in risk of coronary artery disease in younger women who underwent oophorectomy and received estrogens is also consistent with the timing hypothesis (9).

Subgroups analysis of the WHI, focused on the age issues (10) and nonadhering patients (11), provide some support for the timing hypothesis. Examining Relative Risks (RR) first, in the conjugated equine estrogens (CEE)-alone arm, a non-significant (p=0.12) trend toward a reduction in CHD in women younger than age 60 years was observed, this was not evident in the women older than 60 years (i.e. RR, 0.63; CI, 0.36-1.09 for ages 50-59 year). This trend was not apparent (p=0.70) in the CEE plus MPA study (i.e. RR, 1.29; CI, 0.79-2.12 for ages 50-59 year). A similar non-significant (p=0.15) trend toward a reduction in the RR of CHD only in the women less than 10 year since menopause also was observed in the CEE-alone arm. However, a significant increase in the RR of CHD with greater time since menopause was observed in the CEE plus MPA arm (p=0.05). A significant increase in the number of CHD events per 1000 women per 5 year with increasing age was noted in the CEE plus MPA arm, although no such trend was observed in the CEE-alone arm. Similarly, a significant increase in the number of CHD events per 1000 women per 5 year was also observed with greater time since menopause in the CEE plus MPA study, with no significant effect in the CEE-alone arm. These subgroup analyses support the hypothesis that timing of initiation can influence the effects of HRT with either beneficial or neutral effects in younger, more recently menopausal women or harmful effects in older women with longer duration of menopause (4).

It is clear that there is no a single randomized clinical trial that could answer all questions about a given intervention and thus many questions remained after the completion of the WHI. As an example, it remains unclear the cardiovascular effects of HRT administered in lower doses, or by transdermal rather than oral routes of delivery, (the WHI trial used only oral administration and the same dose for all the participants). It is also unclear if formulations containing different estrogens and/or progestogens would be more effective, (not only like WHI that used CEE plus MPA) or with the progestogen given cyclically rather than continuously. The effect of duration of therapy also remains uncertain.

Moreover, although oral estrogen exerts a more favorable influence than transdermal estrogen on traditional cardiovascular risk factors such as high- and low-density lipoprotein-cholesterol levels, recent studies have indicated that oral estrogen adversely influences many emerging risk factors in ways that are not seen with transdermal estrogen. Oral estrogen significantly increases levels of acute-phase proteins such as C-reactive protein and serum amyloid A (12); procoagulant factors such as prothrombin fragments 1+2 (13); and several key enzymes involved in plaque disruption, while transdermal estrogen does not have these adverse effects (14).

Different studies have indicated that transdermal administration of estrogens is safer in regard to the risk of stroke (15) and venous thromboembolism (16), considering the first-pass effect in the liver as an important factor in the prothrombotic impact of oral estrogen. More recently, a case-control study compared transdermal and oral hormone therapy and concluded that, compared with an increased risk of stroke with oral therapy, there was no increased risk with trandermal treatment at a dose of 50 ug or less (17).

Furthermore, very recently some researchers from the WHI Study (18), reported data on post intervention outcomes through a mean of 10.7 years of follow-up among women with prior hysterectomy. Over the entire follow-up, lower breast cancer incidence in the CEE group persisted and was 0.27% compared with 0.35% in the placebo group (HR, 0.77; 95% CI, 0.62-0.95). Health outcomes were more favorable for younger compared with older women for CHD (P=.05 for interaction), total myocardial infarction (P=.007 for interaction), colorectal cancer (P=.04 for interaction), total mortality (P=.04 for interaction), and global index of chronic diseases (P=.009 for interaction). They concluded that among postmenopausal women with prior hysterectomy followed up for 10.7 years, CEE use for a median of 5.9 years was not associated with an increased or decreased risk of CHD, deep vein thrombosis, stroke, hip fracture, colorectal cancer, or total mortality and a decreased risk of breast cancer persisted.

The arrival of lower-dose estrogen therapies, with efficacy in treating vasomotor symptoms and preventing bone loss but with lower risk of bleeding, venous thromboembolic phenomena and undesired effects on vascular integrity promises good results. A significant proportion of the older female population has hypertension and this has been shown to be one of the most important determinants of cardiovascular risk in women. Well-planned controlled trials are needed to examine the effects of this agent on hard cardiovascular outcomes.

In order to answer these controversies, two ongoing

clinical trials have been developed to provide information regarding the role of oral versus transdermal estrogen in younger postmenopausal women, KEEPS (Kronos Early Estrogen Prevention Study) and ELITE (Early versus Late Intervention Trial with Estradiol).
KEEPS Study is a currently randomized, placebo-controlled, double-blinded, prospective trial of the effects of menopausal hormone therapy on subclinical atherosclerosis in recently menopausal women (42-58 years old). The KEEPS is designed to explore the hypothesis that early initiation of hormone therapy, in women who are at the inception of their menopause, will decrease the rate of accumulation of atherosclerotic plaque, indicating a likely delay in the onset of clinical cardiovascular disease.
The ELITE Study enrolled postmenopausal women according to their number of years since menopause, less than 6 years or 10 years or more, to receive either oral 17B-estradiol 1 mg daily or a placebo and vaginal progesterone to those women with intact uterus. The primary hypothesis to be tested is that 17β-estradiol (estrogen) will reduce the progression of early atherosclerosis if initiated soon after menopause when the vascular endothelium (lining of blood vessels) is relatively healthy versus later when the endothelium has lost its responsiveness to estrogen.
Once these studies will be released we will know the real effect, beneficial or not, of estrogen on post menopausal women CVD.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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The effects of a "rediscovered" substance – namely ß-Ecdysone

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In recent years obesity has reached dramatic dimensions and the obese are more likely to develop arteriosclerosis, with the consequence of increased instances of heart attacks and strokes. Furthermore, they may develop type 2 diabetes and suffer from loss of bone mass (osteoporosis), loss of joint cartilage (osteoarthritis, formerly called arthrosis) and loss of muscle mass (sarcopenia).

For the human, obesity is defined as a body mass index (BMI) larger than 30. The BMI, however, is no longer used by clinical scientists because it does not account for differences in fat or muscle mass. The waist circumference as an index of obesity is now used because it determines whether a person is in danger of suffering from the above outlined diseases. It is generally accepted that 2 types of fat distribution exist: The "pear type" is characterized by a fat distribution around the gluteal and thigh area and this type bears no health risks. People with an "apple type" of fat distribution have a high fat load in the abdomen which endangers them to become seriously ill. The Abdominal (visceral) adipocytes secrete so called cytokines which are also secreted during inflammatory processes, hence overweight person of the "apple-type" are in a chronic inflammatory state with higher than normal levels of circulating cytokines, primarily tumor necrosis factor a (TNFα), interleukin 6 (IL6), leptin, adiponectin and others (1,2) which cause increased levels of the marker for inflammation, i.e. C-reactive protein (CRP).

These cytokines have many adverse effects. They increase cholesterol production, particularly the unhealthy low density lipoproteins (LDL) and the very unhealthy triglycerides (3). Furthermore they cause high oxidative stress conditions (4). Altogether, this results in arteriosclerosis with the consequence of increased heart attacks and strokes. Additionally, the high amounts of circulating cytokines result in a desensitization of insulin receptors which finally leads to type 2 diabetes and an even higher risk of arteriosclerosis (5). Taken together, this often leads to immobility.

With increasing age, in women augmented postmenopausally, fat tissue accumulates markedly in bone marrow and in joint fat pads (6,7). These accumulating bone marrow and joint adipocytes have similar properties as the visceral fat cells because they also secrete cytokines causing a local chronic inflammatory state in bone and joints (8,9) and which inhibits the activity of bone and joint cartilage forming cells and stimulates the activity of bone and cartilage digesting cells. This resulted in loss of bone mass (osteoporosis) and joint cartilage tissue (osteoarthritis). In addition, due to mechanical compression being overweight is harmful for knee and hip joints.

The increase of obesity is primarily the result of unhealthy nutrition, too much fat rich fast food and too many carbohydrate rich soft drinks. Even though attempts were made to improve nutritional habits of the population, this did not change the increased incidence of obesity. Therefore, affordable dietary supplements of plant origin which inhibit cholesterol and triglyceride production and fat cell formation would be desirable and - if existent - introduced to prevent obesity and the resulting diseases.

In Ayurveda Medicine Guduchi was shown to prevent obesity. A search for an active compound yielded the presence of ß-Ecdysone (Ecd) improves training induced muscle performance and is therefore used by many athletes and body builders in high quantities without any unhealthy side effects.

The ovariectomized (ovx) rat is an ideal model to study obesity, hypercholesterolemia, osteoporosis and osteoarthritis (10-14) and in this animal model we showed that Ecd reduced visceral fat load (Fig. 1a) and serum leptin levels (Fig. 1b). According to the above explained, this reduces cytokine production and the accompanying oxidative stress. Consequently less cholesterol and LDL is produced (Fig. 1c and d) and less LDL is available for oxidation and formation of arteriosclerotic plaques. Hence, if such effects could be confirmed in the human this should result in a reduction of arteriosclerosis and consequently to fewer heart attacks and strokes.

Earlier we showed that Ecd was able to reduce ovx induced loss of bone mineral density and of joint cartilage tissue (14,15). The results from these animal studies encouraged us to test whether Ecd can also reduce the fat load in the bone marrow and in joint fat pads. Indeed, Ecd reduced bone marrow and knee joint fat load significantly and this resulted in less loss of trabecular bone mineral density and of the thickness of articular cartilage tissue.

We tested also whether Ecd has estrogenic or androgenic properties which could explain these favourable results. Neither in receptor binding nor in animal studies did we observe estrogenic or androgenic properties of this steroid (14).

To test whether these experimental data are of value to prevent the Metabolic Syndrome in the human we performed a 3 months lasting open clinical trial to study putatively beneficial metabolic properties of Ecd.

A widely used edible plant, namely spinach contains Ecd. Therefore we used an Ecd enriched spinach powder for our observational study in which we included persons (n=20) with serum cholesterol levels above xx and serum LDL values above xx and with a waist circumference between 90-100cm in women and 100-110cm in men. They received the Ecd enriched spinach powder in capsules which contained 75-100 mg Ecd (VerdeVital GmbH, Göttingen, Germany). After this period serum cholesterol, particularly the LDL was reduced by 13% and triglycerides by 42%. Total body weight was reduced by 1.5kg, body fat mass by 5% and most importantly the waist circumference by 2.5cm. Muscle mass increased by 4%. Other parameters like liver enzymes and white and red blood cell counts remained unchanged.

On the basis of these experimental and clinical results we conclude that the Ecd enriched spinach extract may well be suitable to prevent the occurrence or progression of obesity and the subsequent diseases with all their health threatening consequences like arteriosclerosis, osteoporosis and osteoarthritis.

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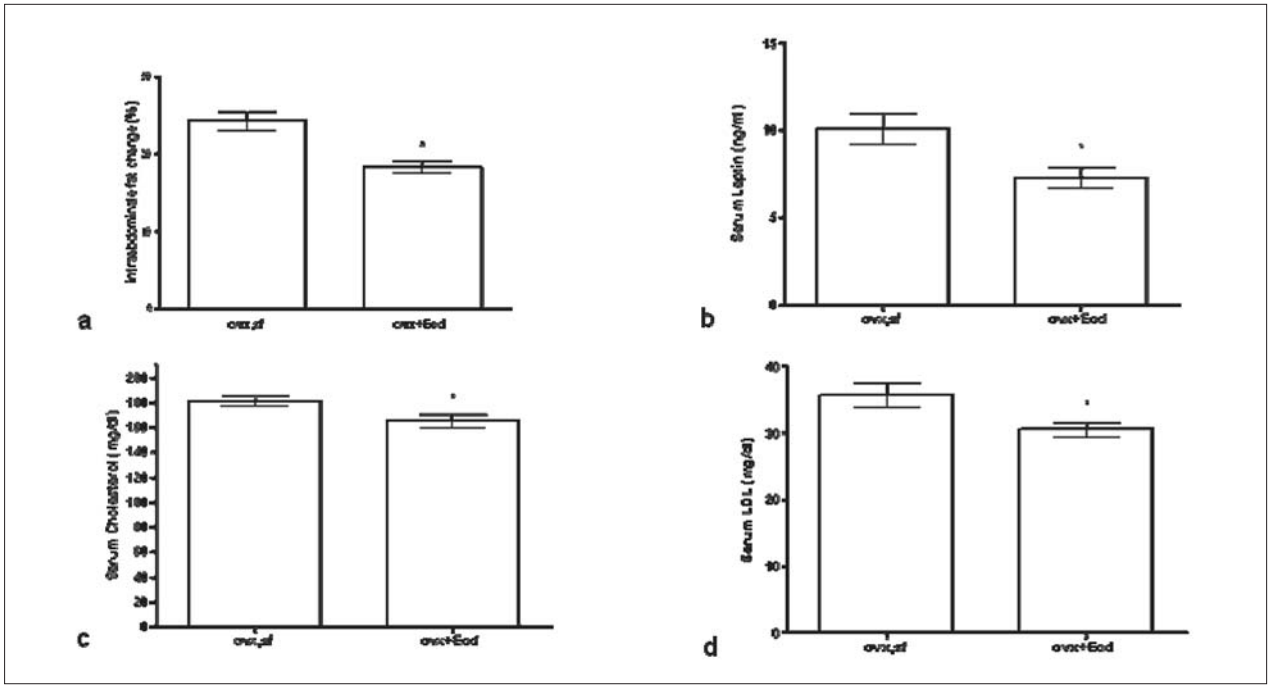

Fig. 1 - In ovx rats abdominal fat load (a), serum leptin (b), cholesterol (c), LDL (d) are significantly reduced by Ecd.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Future of fertility preservation

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Introduction

Due to remarkable advances in the treatment of cancer, we have seen great improvements in long-term survival rates of pediatric and reproductive-age male patients. Unfortunately, fertility in adult life might be severely impaired by these treatments. A number of fertility preservation options now exists that may allow patients to conceive once they have overcome their disease. For females, embryo and oocyte cryopreservation are already routinely applied, but cryopreservation of ovarian tissue is the only option available for prepubertal girls, women without partners and those who cannot delay the start of chemotherapy. Orthotopic reimplantation of frozen-thawed postpubertal ovarian tissue has proved effective (1), with the birth of close to 20 healthy children. However, pregnancy has not yet been achieved with transplantation of ovarian tissue from prepubertal girls and the global success rate is unclear, since the number of transplantations performed, is unknown. Other approaches, such as follicle isolation with subsequent-in vitro maturation (IVM), are currently under investigation.
In males, postpubertal patients (from age 12 upwards) can benefit from sperm cryopreservation and healthy live births have been reported with frozen-thawed sperm stored for up to 28 years (2), but little is known about the effects of gonadotoxic treatments on the prepubertal testis. While differentiating spermatogonia are extremely susceptible to cytotoxic agent damage, the less active stem cell pool may also be depleted, resulting in loss of spermatogonial stem cells (SSCs) that normally differentiate to produce sperm after puberty (3).
For prepubertal boys, preservation of SSCs is a potential, albeit still experimental, strategy for preserving fertility in the hope that future technologies will allow its safe use (4). Although clinical experience of immature testicular tissue (ITT) storage has already been published (6), successful fertility restoration with frozen-thawed ITT in humans has not yet been reported. Cryopreservation techniques for ITT and potential fertility restoration approaches using ITT will be reviewed.

Fertility preservation with ITT

SSCs can either be cryopreserved as a cell suspension or in the form of tissue. However, preparation of cell suspensions may compromise cell survival (6) and requires tissue digestion, suppressing cell-to-cell interactions necessary for cell proliferation and differentiation. Tissue cryopreservation must strike a balance between optimal conditions for each cellular type and problems may arise from insufficient solute penetration in highly compacted tissue and formation of extracellular ice.
Three teams have reported slow-freezing protocols for prepubertal human ITT that have yielded good structural integrity and spermatogonial recovery (7-9). Further evaluation of the functional capacity of cryopreserved human ITT in a xenotransplantation model showed that spermatogonial cells were able to proliferate, although a high proportion were lost and abnormal spermatogenic differentiation was observed (10). Vitrification (V) might constitute a better approach, preventing ice crystal formation by use of high concentrations of cryoprotectants and ultrafast cooling velocity. Its potential was recently demonstrated in non-human primate and human ITT, achieving good seminiferous tubule (ST) integrity, survival and proliferation of spermatogonia (11,12).

Fertility restoration after ITT cryopreservation

In the light of results obtained from animal studies, frozen SSCs may provide some hope of fertility restoration in prepubertal boys. Three approaches may be considered: transplantation of purified cell suspensions back to their own testes, autografting of testicular pieces, testicular cell aggregates or whole testes, or IVM up to a stage at which they are competent for normal fertilization through intracytoplasmic sperm injection (ICSI). However, none of these methods have proved efficient or safe in humans as yet.

Spermatogenesis could be reinitiated after transplantation of isolated testicular stem cells to germ cell-depleted testes (13). SSCs relocate from the lumen onto the basement membrane of STs, self-renew and produce differentiating daughter cells. Autologous SSC transplantation has proved successful in a number of species, including monkeys (14), and yielded healthy progeny (15-18). In humans, preclinical in vitro studies have demonstrated the feasibility of transplanting germ cell suspensions into testes (19).

Testicular tissue grafting involves transplantation of SSCs with their intact niches. Complete spermatogenesis following grafting has been reported in a number of species, including non-human primates (20), and haploid germ cells isolated from mouse testis homografts and rabbit testis xenografts have been used with ICSI to generate offspring (21).

Transplantation of testicular cell aggregates is challenging, since the different cell types have to form functional three-dimensional cell associations after transplantation in order to produce a supportive microenvironment for spermatogenesis. Nevertheless, complete spermatogenesis has been observed (22-26) and mouse offspring obtained (24). This technique offers the possibility of using cell sorting methods before grafting and could therefore be beneficial from a clinical perspective when testicular tissue is potentially contaminated with cancer cells (27).

The first convincing demonstration of human testis transplantation was reported in 1978 (28). However, before this strategy can be considered for fertility preservation purposes, appropriate cryopreservation methods should be developed for whole organs.

IVM of SSCs, yielding in vitro-derived haploid gametes available for ICSI, circumvents the risk of reintroducing malignant cells, making this procedure potentially highly beneficial for cancer patients. Efforts have focused on establishing optimal in vitro culture systems to allow male germ cells to complete meiosis and spermatid elongation in experimental conditions. In mice, IVM recently succeeded in obtaining sperm (29) and even offspring from fresh ITT, as well as complete spermatogenesis in frozen ITT (30). In humans, a number of studies have investigated culture systems suitable for in vitro spermatogenesis, but the whole process has not yet been reproduced.

Resolving numerous technical issues should lead to safe and efficient methodologies for fertility restoration after storage of preserved gametes from today's prepubertal patients, which will allow them to consider fertility restoration options that will emerge in the next 20-30 years.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

A high dose intravenous immunoglobulin therapy for women with four or more recurrent spontaneous abortions

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Introduction

Recurrent spontaneous abortion (RSA) is defined as the loss of three or more consecutive pregnancies in the first trimester (1). The RSA affects 1-1.8% of women (1,2). A wide variety of causes participate in the pathogenesis of RSA, including uterine anomalies, cervical incompetence, autoimmune diseases, antiphospholipid antibody, chromosomal abnormalities of couples, thrombophilic disorders, endocrinological abnormalities, and microbial infections (2,3). For example, parental chromosome abnormalities represented by balanced type translocations are associated with approximately 4% of couples with RSA compared with 0.2% in normal population (4). However, the etiology in approximately 50% of RSA is unknown, therefore designated as unexplained RSA. It is postulated that they have immunological etiologies (1).

The precise mechanism underlying the pathology of RSA remains poorly understood. In this context, no standard therapeutic modality for unexplained RSA have been established so far, despite several lines of evidence indicating some therapeutic efficacy of unfractionated heparin or low molecular weight heparin with or without low dose aspirin, paternal lymphocyte immunization, intravenous immunoglobulin (IVIg), predonisolone, and progestin (5-7). In the recently emerging literature, novel clinical approaches with the use of tumor necrosis factor inhibitors (8,9) and granulocyte colony-stimulating factor (10) have been conducted for the treatment of RSA.

We for the first time developed a high dose intravenous immunoglobulin therapy (HIVIg) during early gestation for severe cases with RSA of unexplained etiology in 1993 and previously reported the efficacy in a preliminary study (11).

Materials and methods

Patients

This prospective study was performed as a multi-center study in Japan, and conducted with informed consent from all of the subjects. The study was approved by the institutional ethical boards of the Kobe University Hospital and the Hokkaido University Hospital. During the period between 1993 and 2010, RSA women were admitted to the study if they met all of the following requirements: Subjects must have (i) a history of four or more consecutive spontaneous abortions in the first trimester, (ii) unexplained etiology of RSA, and (iii) no allergy for immunoglobulin or IgA deficiency disease.

All patients underwent several examinations of ultrasound, hysterosalpingography, endometrial biopsy, and conventional blood analyses for RSA screening, and were diagnosed as having RSA of unexplained etiology. The blood analyses included chromosome karyotypes of couple; measurements of progesterone in mid-luteal phase, prolactin, thyroid, liver, kidney functions, hemostatic coagulation factors such as d-dimer, factor XII, protein C, protein S; and autoimmune factors such as antinuclear antibody, complements, anticardiolipin, β2-glycoprotein I-dependent anticardiolipin antibodies, and lupus anticoagulant.

Immunoglobulin therapy

They underwent HIVIg therapy (intact type immunoglobulin 20 g daily in the course of 5 days; a total dosage of 100 immediately after a gestational sac was detected in the uterus by ultrasound. Medications of intact type immunoglobulin including Venoglobulin-IH (Benesis, Osaka), Kenketsu glovenin-I (Nihon

Parmazeutical Co., Tokyo) and Sanglopor (CSL Behring, Tokyo) were used.

Results

We had conducted HIVIg therapy in 60 RSA women with the ages ranging from 24 to 44 years old who had a history of 4 to 8 spontaneous abortions, and confirmed pregnancy outcome (Table 1). The live birth rate was 73.3% (44/60). Fifteen pregnancies ended in spontaneous abortion, and one ended in intrauterine fetal death at 31 weeks of gestation due to severe pregnancy induced hypertension and sudden abruptio placenta. The 15 abortions consisted of 2 spontaneous abortions of a fetus with normal chromosome karyotype, 11 spontaneous abortions of a fetus with abnormal chromosome karyotype (SAAK), and 2 with unknown karyotype. It was impossible to assess the efficacy of HIVIg among the 11 SAAK fetuses who were destined to die. If the 11 pregnancies resulting in SAAK were excluded, the live birth rate was high as 89.8% (44/49).

Pregnancy complications including premature delivery (18.2%) and intrauterine fetal growth restriction (13.6%) were observed. Fetal anomaly of cleft lip was seen in one case. The adverse effects of HIVIg in the mothers including rash/fever (13.3%) and the elevation of d-dimer levels (6.7%) were found.

Discussion

It is well acknowledged that intravenous use of a high dose of immunoglobulin (HIVIg) is practically effective, and this therapy has long been applied to a wide variety of immune-mediated diseases such as idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, Kawasaki's disease, and myasthenia gravis (12,13). Several mutually non-exclusive mechanisms of action, which include the suppression of inflammation and modification of Fc receptor, T cell, B cell or macrophage functions, are proposed to account for the immunoregulatory effects of the HIVIg therapy (13).

To assess the efficacy of IVIg in women with unexplained RSA, randomized, double-blind, and placebo-controlled trials with use of a medium dose of IVIg therapy, in which 20-40 g of Ig/person is infused weekly or every 2-4 weeks during early and mid-gestation, have been performed in 1990s (14-19). Conclusions drawn from these IVIg trials are controversial. Thereafter, reports of meta-analysis (20) and systematic review (21) concerning efficacy of IVIg therapy suggested that a medium dose of IVIg was effective among women with secondary RSA. On the other hand, our group for the first time tried HIVIg therapy for severe cases of unexplained RSA, in which 100 g of intact type immunoglobulin was infused intravenously over the course of 5 days at approximately 5 weeks of gestation. We previously reported high live birth rates among small-scale subjects in the preliminary HIVIg studies (11,22). Thereafter, the study was continued and consequently we confirmed the high birth rate and the efficacy of HIVIg for severe cases of unexplained RSA in the present study. HIVIg therapy was well tolerated in most of these subjects, and none of them discontinued HIVIg therapy due to serious adverse effects.

Several immunological mechanisms underlying the pathophysiology of RSA and action of IVIg have been suggested. IVIg modulate NK cell dynamics and cytokine production. The immunomodulatory effects of IVIg on NK cells in RSA women include the reduction of peripheral NK cell number and cytotoxicity (22-26) and the increase in expression of inhibitory receptor CD94 on NK cells (27). IVIg therapy in RSA women was shown to drive a Th1/Th2 balance toward Th2 shift (28,29) and to increase serum cytokine (29) and G-CSF levels (30).

Using a mouse model of immunological reproductive failure, we for the first time demonstrated that a high dose of intact type- immunoglobulin but not Fab-immunoglobulin restored the fecundity and that spleen cells adoptively transferred from im-

TABLE 1 - PREGNANCY OUTCOME, COMPLICATIONS AND ADVERSE EFFECTS IN 60 WOMEN WITH SEVERE CASES OF UNEXPLAINED RECURRENT SPONTANEOUS ABORTION (RANGE 4-8 ABORTIONS) WHO UNDERWENT A HIGH DOSE INTRAVENOUS IMMUNOGLOBULIN THERAPY.

Pregnancy outcome	Number	Complications	Number (%)
Live birth	44	Premature delivery	8/44 (18.2)
IUFD at 31 weeks	1	IUGR	6/44 (13.6)
Spontaneous abortion	15	Anomaly (cleft lip)	1
Normal chromosome karyotype	2	Adverse effects on mothers	
Abnormal chromosome karotype	11	Rash and fever	8/60 (13.3)
Unknown karotype	2	Elevation of d-dimer	4/60 (6.7)

IUFD= intrauterine fetal death; IUGR= intrauterine growth restriction.

munoglobulin injected nonpregnant donors to pregnant recipient mice of reproductive failure completely restored the fecundity. The restoration of the fecundity was concomitant with reduction of TNF-α and IFN-γ expressions in the placentas. CD11b+ macrophages transferred from donor mice accumulated selectively in the placentas of recipient mice, suggesting that macrophages as mediator and/or effector cells may play a key role in mechanisms of HIVIg efficacy (31). These observations may explain a favorable pregnancy outcome in HIVIg treated RSA women who have immunological abnormalities. These data with use of HIVIg strongly support the evidence showing the usefulness of this therapy as immune modifier, when performed during early gestation. It is likely that HIVIg correct etiological immunological abnormalities in RSA women. Figure 1 shows hypothetical mechanisms of immunoglobulin-mediated prevention of spontaneous abortion.

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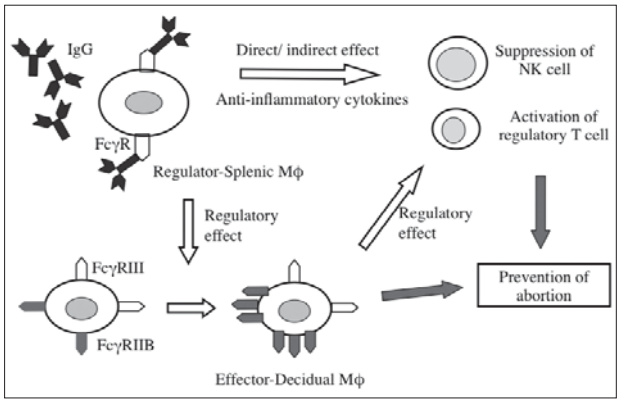

Fig. 1 - Hypothetical mechanisms of immunoglobulin-mediated prevention of abortion.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Using the IVM/IVF model for characterization and validation of the human ovulatory cascade

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Introduction

Reproduction is a major area of research since the onset of biological science. However, the human female reproduction research lags substantially behind mainly due to the lack of proper biological materials and the lack of proper tools. Therefore, many ovulatory processes that were described at the molecular levels, mainly in rodents, were not validated in human.
Ovarian follicular development and ovulation in mammals is a highly regulated process. This process involves the selection of a dominant follicle, reactivation of oocyte meiosis, rupture of the follicle wall, cumulus expansion and tissue remodeling to form the corpus luteum. These processes are fundamental to successful establishment of pregnancy, but importantly also impact on the developmental potential of resultant embryos. Genes involved in this processes, have been the subject of growing interest (1,2).
The LH surge, derived from the pituitary, is the single trigger, initiating the cascade of events, leading to ovulation. Elucidation of these fascinating processes and understanding the molecular regulation of the human ovulatory cascade is of critical importance.
The main events resulting from the LH surge are cumulus expansion, oocyte maturation, follicular rupture, and luteinization, all of which are essential for proper reproduction.
In response to the LH surge, various changes in gene expression and follicular structure occur in the theca, granulosa, cumulus and oocyte compartments of the ovarian follicle. Systemic and local inputs co-ordinate with signals from the oocyte, Thus ovulation is under complex control facilitating synchronization of oocyte maturation with its release and permitting the selection of oocytes with full developmental competence (3).
Traditionally, animal models have served for the study of human biological processes (4). Animal models have several important advantages including availability, low variability, genetic homogenicity, use in time-course experiments and transgenic and knock-out models. In fact, it will be reasonable to say that research on animals has taught us nearly all we know about cell biology (4,5).
However, animal models have also several disadvantages including the variability from humans and the ethical controversies regarding animal research and their welfare (4,6).
Most of the recent advances in understanding of the molecular basis of folliculogenesis and ovulation have come from rodent models (7). Also a considerable contribution came from studies in domestic animal (8) and primate species (9,10). However, which species are most appropriate model for understanding critical genes and processes in human ovulation is currently unclear.
In the human, like in the mouse, LH and FSH were shown *in-vitro* and in clinical practice to orchestrate the ovulatory cascade. Only few studies in human revealed common mechanisms with animal studies. For example, FSH was shown to induce LHR in mural granulosa cells (11,12), and hCG to induce amphiregulin (Areg) expression and progesterone synthesis (12,13). However, it appears that there are differences in a variety of molecular mechanisms between human and animal models. For example, SULT1E1 was found to be expressed in the rodent ovary and highly regulated during folliculogenesis. Moreover, knock-out mice suffered from impaired ovulation (14). How-

ever, this gene was not found to be expressed in primate and human granulosa cells (personal communication). Another gene, sFRP4 was shown in the mouse to be up-regulated by hCG (15-17) and presumably to inhibit ,-catenine, an inhibitor of the LH pathway (16). We found that sFRP4 in human is down-regulated by hCG both *in vitro* and *in vivo*, while sFRP5 is induced by hCG and may replace the role of sFRP4 as LH mediated beta-catenine suppression in the human ovary (18).

Thus, data obtained from animal studies must be validated in human experiments. However, there are relatively few publications on the molecular mechanism of human ovulatory process which is probably due to ethical and practical difficulties in obtaining relevant samples.

In-vitro maturation of human oocytes (IVM) is an emerging technology with promising potential in specific indication. The main advantage of this technique is the fact that it does not require gonadotropin stimulation, and therefore the treatment is shorter, simpler, less expensive, and most importantly, safer, avoiding the risk of ovarian hyperstimulation syndrome (OHSS). The technique was found to be particularly suitable for women with PCOS and women who are at high risk of OHSS (19,20).

During IVM process, cumulus-oocyte complexes (COCs) are obtained mostly at germinal vesicle (GV) stage and are matured in vitro by maturation medium into metaphase II (MII) stage (21). Human oocyte can resume meiosis, reach metaphase II stage, and by intracytoplasmic sperm injection (ICSI) can be fertilized at high ratio (22). The application of *in vitro* maturation (IVM) of oocytes as a technology to assist clinical infertility treatment remains poor because of the reduced developmental competence of oocytes after IVM. Pregnancy and live birth rates do not match those reported for IVF cycles using full hormonal protocols with triggered maturation in vivo (23). Furthermore, the quality of maturation appears to be suboptimal since embryos resulting from *in vivo* matured oocytes have better developmental capacity when compared with their *in vitro* matured counterparts. Indeed, at least one major hurdle that must be overcome before IVM becomes a mainstream procedure is the handling of immature oocytes (24).

IVM and IVF procedures allow us to obtain biological material from different stages of the follicular development. This includes follicular fluid as well as granulosa cells–mural obtained from the follicular fluid and cumulus obtained during oocyte denudation. During IVM without hCG priming, non-luteinized cells can be obtained. Information from such research is of crucial importance for improving fertility control and treatment (25).

Our aim is to explore the human folliculogenesis and the preovulatory follicular events. Our model, described in this article is based on follicular fluids and granulosa cells obtained during ART treatments.

Gene characterization during different stages of antral folliculogenesis

Previously we generated a murine ovulatory-selective cDNA library (26). In order to examine the presence of these genes and characterize their expression in follicles of different size in human GCs, we used the IVM procedure to obtain mural granulosa cells from small (4-8 mm) and large (10-14 mm) follicles and compare it to mural granulosa cells obtain from the large pre-ovulatory follicles of IVF (25). We have shown the presence of several known ovulatory genes such as StAR, amphiregulin, epiregulin, LHR, cyp19A1 (aromatase) and EPHX1 but also genes that were not shown previously or were shown only recently by us in human ovary such as MAGOH, ELOVL5 (25), sFRP4 (18) and ADAMTS1 (27).

This model system allowed the characterization of ovulatory gene expression *in-vivo* during the different stages of antral folliculogenesis. For example, we found that the expression of sFRP4 was inversely correlated to follicular size: mural GCs obtained from small luteinized IVM follicles expressed higher levels of sFRP4 than mural GCs obtained from large luteinized IVM follicles, and that sFRP4 expression further decreased in mural GCs obtained from preovulatory luteinized follicles (18).

We also have shown that LHR expression was shown to correlate with follicle size. The small follicles had the lowest levels of LHR expression, and these levels were higher in medium-sized follicles, with the highest levels of expression (6-fold those of the small ones) being observed in the large preovulatory follicles (Fig. 1) (28).

IVM protocols without hCG priming allows us to obtain GCs from non-luteinized follicles and compare them to cells from luteinized follicles from IVM. This allows us to study the effect of LH surge *in-vivo* on selected genes. For example, we found that ADAMTS-1 levels in **non-luteinized** mural GCs were undetectable compared to the high expression that were present in **luteinized** mural GCs obtained from follicles of similar size (>10 mm). These results suggest that ADAMTS-1 is an hCG regulated gene in human mural GCs (27).

Ovulatory gene expression in luteinized versus non-luteinized GC in cell culture

Culture of granulosa cells obtained during ART procedures allows us to study ovulatory gene expression fol-

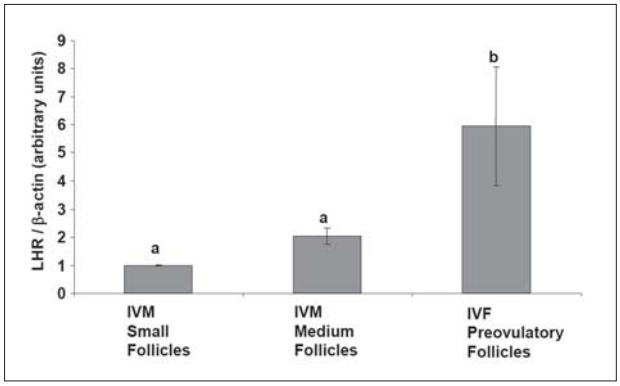

Fig. 1 - Luteinizing hormone receptor (LHR) expression pattern in mural GCs. Total mRNA of mural GCs was purified from small follicles (<10 mm), large follicles (10-14 mm) aspirated during human chorionic gonadotropin (hCG)-primed in vitro maturation (IVM) procedures and preovulatory follicles (>17 mm) aspirated during in vitro fertilization (IVF) procedures. The RNAs were subjected to reverse transcription and quantitative real-time polymerase chain reaction with LHR and â-actin (loading control) primers. LHR expression was calculated relative to the â-actin level in the same sample. The results are expressed as mean±SEM of three independent experiments (3-5 patients in each group) Values with different superscripts are significantly different (*P*<0.05). Reprinted from Maman et al. 2012 (28).

lowing treatment with various stimulants and inhibitors thus enabling us to further characterize ovulatory pathways in humans.

Luteinized GCs for culture are relatively easy to obtain during IVF treatment cycles and were used by many researchers in the past 20 years. However, there is a concern regarding the use of luteinized GCs in cell culture to study molecular ovulatory processes. Following the LH surge and luteinization there might be major differences in the cellular mechanisms and response to various stimuli of luteinized cells as compared to non-luteinized cells.

It was previously published that granulosa cells regain their responsiveness to gonadotropin stimulation following culture in hormone-free environment for 3-7 days (29) but there are only few published papers using non-luteinized GC (12) and regarding the use of luteinized GC compared to non-luteinized GC (11).

IVM protocols without hCG priming allows us to obtain GCs from non-luteinized follicles and compare them to cells from luteinized follicle in cell culture. Based on published data we established a culture protocol of luteinized GC for 48 hours in culture media without gonadotropins.

We used non-luteinized mural GCs and luteinized GC in cell culture and examined the expression of several ovulatory genes following treatment with hCG and FSH. We show the expression of sFRP4 in cultured GC. We found that the expression pattern of sFRP4 in luteinized and non-luteinized cells in response to stimulus by hCG was similar, thus supporting the use of luteinized GCs as a model system to investigate ovulatory processes (25).

In order to examine LHR functionality in cumulus cells, the expression of genes known to be up-regulated after LH surge (30) was evaluated. Cumulus GCs were cultured and stimulated with hCG and harvested for qPCR 8 h later. The results showed that Has-2, PTX-3, and TSG-6 were induced about 2-, 1.5-, and 5-fold, respectively, after hCG administration (Fig. 2) (28).

Correlation of selected cumulus genes to oocyte maturation, fertilization capacity and pregnancy

Correlating gene expression in cumulus cells from individual COC to oocyte competence and fertilization outcome can usefully serve as a high throughput and non-invasive means for the assessment of oocyte quality, embryo competence and pregnancy outcome. This technology using quantitative RT-PCR can help to define the potential biomarkers correlated to a competent oocyte and also improve the selection of healthy embryos leading to pregnancy. Increasing number of papers have shown correlation of cumulus gene expression with oocyte maturation (31,32), fertilization rate (31) and pregnancy outcome (32-34), McKenzie et al. have shown that the expression levels of GDF9 targets in cumulus cells of individual oocytes has a correlation to embryo quality (31). Assou et al. employed a robust multiple microarray approach to identify novel cumulus genes as biomarkers for embryo and pregnancy outcome (33). They identified 45 genes as biomarkers and demonstrated a good correlation between the expression profile of these genes and pregnancy outcome without a relationship to the morphological grade of the embryos (33).

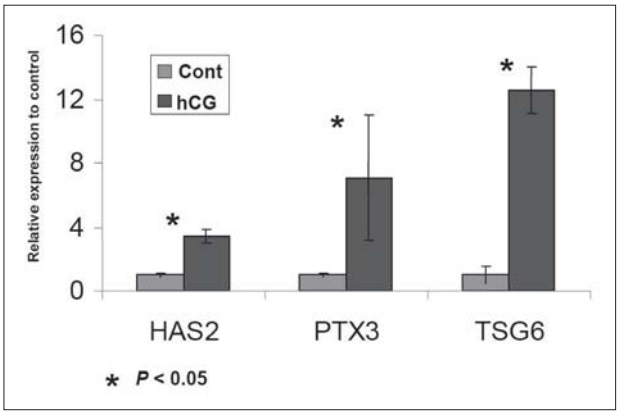

Fig. 2 - Expression of cumulus expansion genes. Cumulus cells from preovulatory follicles were cultured and supplemented with human chorionic gonadotropin (hCG) 1U/ml. After 8 h total mRNA was purified and subjected to reverse transcription and qRT-PCR with HAS2, PTX3, TNFAI6 and β-actin (loading control) primers. The results are expressed as mean±SEM of three independent experiments. Reprinted from Maman et al. 2012 (28).

We have previously shown that ADAMTS-1 and sFRP4 can serve as a potential biomarker for oocyte quality. We found that sFRP4 expression in cumulus GC is inversely correlated to oocyte maturation, with the highest expression in the cumulus of GV oocytes compared to that of MI oocytes and the lowest expression in the cumulus of MII oocytes (18). Also we found that ADAMTS-1 expression in cumulus GC is positively correlated to oocyte fertilization capacity (27).

We also investigated the correlation between the levels of LHR expression in cumulus GCs and the related oocyte maturation stage and shown that the highest level of expression in cumulus GCs obtained from metaphase II (MII) oocytes and lowest levels in cumulus GCs obtained from immature oocytes (germinal vesicle [GV] and metaphase I [MI]) (28). These results clearly demonstrated that LHR expression in cumulus GCs correlated with the oocytes' maturational stage (Fig. 3).

LHR expression in cumulus GCs of MII oocytes obtained during IVF procedures at the time of oocyte denudation was also investigated. Cumulus GCs were then grouped based on related oocyte fertilization outcome and subjected to RT-PCR. We found significantly lower levels of LHR mRNA in the cumulus of oocytes that underwent fertilization compared to oocytes that failed to be fertilized (28), providing evidence that LHR expression in human cumulus GCs can be a marker for oocyte quality and capacity to be fertilized. High expression of LHR correlated with lower fertilization capacity (Fig. 4).

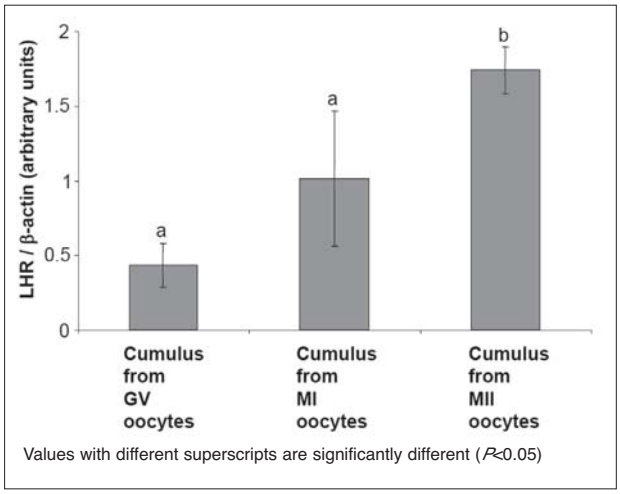

Fig. 3 - Luteinizing hormone receptor (LHR) expression in cumulus granulosa cells (GCs) according to oocyte maturation stage. Cumulus GCs obtained during intracytoplasmatic sperm injection (ICSI) after in vitro fertilization procedures were sorted according to maturational stage of related oocytes, germinal vesicle (GV), metaphase 1 (MI) and metaphase 2 (MII). Total mRNA was purified from grouped cumulus GCs of each category and subjected to reverse transcription and quantitative real-time polymerase chain reaction with LHR and â-actin (loading control) primers. The results are expressed as mean±SEM of three independent experiments, 3-5 cumulus GCs in each category (values with different superscripts are significantly different, *P*<0.05). Reprinted from Maman et al. 2012 (28).

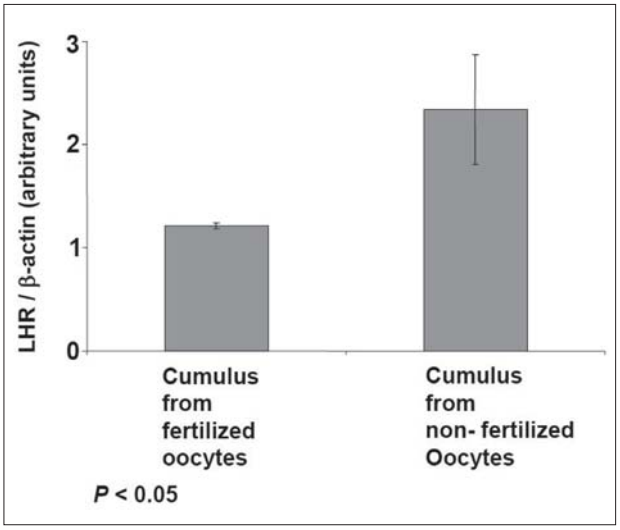

Fig. 4 - Luteinizing hormone receptor (LHR) expression in cumulus granulosa cells (GCs) according to fertilization capacity. Cumulus GCs were collected from an MII oocyte during an ICSI procedure and grouped according to the fertilization fate of the related oocyte. Total mRNA was purified from grouped cumulus GCs of each category and subjected to reverse transcription and quantitative real-time polymerase chain reaction with LHR and â-actin (loading control) primers. The results are expressed as mean±SEM of three independent experiments, 3-5 cumulus in each group (*P*<0.05). Reprinted from Maman et al. 2012 (28).

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ORAL PRESENTATIONS AND POSTERS

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Thyroid and parathyroid dysfunction as a risk factor for osteoporosis in women

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Introduction

Osteoporosis is a metabolic bone disease caused by a reduction of bone mineral density and disruption of bone strength (1). The substance of the bone is reduced with ageing thus increasing the risk of fracture (2). Hormonal dysfunction during ageing may influence the onset and manifestation of osteoporosis.
Demographic calculations in Serbia predict that the 21st century will be the century of the elderly (3). This may be true for other countries as well, thus placing senile osteoporosis as a major health burden. The etiology of senile osteoporosis is multifactorial. Genetic predisposition (4), low vitamin D levels, malabsorption, low estrogen and androgen levels, thyroid and parathyroid disfunction have cumulative influence on bone structure (5). During the ageing process biologically active parathormone (PTH) concentration increases (6), inducing increased bone turnover, mainly through osteoclast's bone resorption. Thyroid dysfunction is not uncommon in the elderly and often goes clinically unrecognized (7).
The aim of this study was to assess thyroid and parathyroid function in elderly women with osteoporosis.

Material and metods

A group 260 elderly women referred by GP for Dual photon Absorptiometry (DEXA) to the Osteoporosis Unit, Division of Endocrinology, Diabetes and Metabolism, Department of Medicine of the "Zvezdara" University Medical Center, were studied. All of them have never been previously treated for osteoporosis and have never done a DEXA examination. Bone mineral density of the lumbar spine and hip was assessed by the Hologic Explorer. T score, Z score and bone mineral density (BMD) were recorded. After obtaining an informed consent, assessment of thyroid and parathyroid function was done. It included analysis of thyroid stimulating hormone (TSH), free thyroxine 4 (FT4), thyroglobulin antibodies (TgAb), thyroid peroxidase antibodies (TPOAb), parathyroid hormone (PTH), s-calcium and s-phosphorus.
Measuring the concentration of TSH (ultrasensitive method), FT4, TPOAb, TgAb, PTH, were performed using CLIA (chemiluminescent immune determination), the camera automatically determine the "Immulite 1000". TSH has been expressed in mU/L, with reference values from 0.4 to 4.0 mU/L. Values lower than 0.4 mU/L characteristic for hyperthyroidism, while values greater than 4.0 mU/L are characterized by hypothyroidism, subclinical or manifest primary hypothyroidism. FT4 has been expressed units of pmol/L, with reference physiologic values from 12.4 to 24.4 pmol/L. TgAb and TPOAb have been expressed in IU/mL, with reference values 0-10 IU/mL. PTH has been expressed in pg/ml with reference values for adults 10-65 pg/ml.
All results were expressed as mean ± standard error. A level of significance was set at $p>0.05$. Linear correlation was used to assess the influence of hormone levels on DEXA parameters. The study was approved by the "Zvezdara" Ethics Committee.

Results

In the group of 260 women, 20 patients had diabetes mellitus, 1 had pancreatitis with malnutrition, 5 had previously received glucocorticoid therapy and 3 were

on estrogen therapy. Osteopenia was present in 36 subjects. This left 195 women with osteoporosis that were included in the study. The mean age of 195 women with osteoporosis was 72±0.40 years. However, 11 women did not show up for hormonal analysis. Hence, statistical analysis was done in the group of 184 women.

Parathormone disorder was found in 58% of participants. Mean PTH level in this group of women was 110.97±5.15 pg/ml. Elevated PTH levels showed a significant positive correlation with decrease of T score levels (- 3.12 ± 0.04, r:-0.94, p<0.01), Z scores (-1.38 ± 0.06, r:-0.82, p<0.01) and BMD (0.56±0.01 gr/cm^2, r=0.93, p<0.01).

Thyroid disorder was present in 31% of participants, unaware of thyroid illness (Table 1). Decreased TSH levels, with the mean of 0.13±0.02 mU/l, indicating hyperthyroidism, were found in 31 women. A significant correlation was found between decreased TSH levels and decreased T score (-3.1 ± 0.11, r = 0.25, p<0.01), Z score (-1.42 ± 0.15, r= 0.51; p<0.01) and BMD (0.55 ± 0.03 gr/cm2, r = 0.92, p<0.01). Elevated TSH levels with the mean of 14.65 ± 3.2 mU/l, indicating hypothyroidism, were found in 26 women. A significant correlation was found between elevated TSH levels and decreased T score (-3.15 ± 0.1, r = 0.69; p<0.001), Z score (-1.51 ± 0.12, r=-0.62; p<0.01) and BMD (0.56 ± 0.02 gr/cm2, r = 0.92, p<0.01). The analysis of TPOAb, TgAb confirmed that 20 patients with hypothyroidism and 15 with hyperthyroidism had autoimmune thyroid disease.

Discussion

Our study has shown that thyroid and parathyroid dysfunction are not uncommon in women with senile osteoporosis. They influence bone metabolism, contributing to low bone mineral density. Their treatment would improve therapeutic outcome of osteoporosis.

Risk of osteoporotic fractures throughout the life period is estimated at 30-40%. It is believed that 30-50% of women and 15-30% of men have an osteoporotic fracture during their lifetime. The prevalence of osteoporotic fractures in Serbia is unknown. But it is expected to increase dramatically with the aging of the population, according to demographic calculations in Serbia (3).

Table 1 - BONE QUALITY IN SUBJECT WITH THYROID DYSFUNCTION.

Parameters	Hyperthyroidism	Euthyroidism	Hypothyroidism
TSH	0.13±0.02	1.58±0.07	14.65±3.2
FT4	24.48±2.46	16.8±0.45	12.57±0.86
T *score*	-3.2±0.11	-3.16±0.05	-3.15±0.1
Z *score*	-1.42±0.15	-1.41±0.06	-1.51±0.12
BMD	0.55±0.03	0.58±0.01	0.61±0.02
number	31 (17%)	127 (69%)	26 (14%)

Osteoporosis can be primary (menopausal, senile, idiopathic) or secondary. Secondary osteoporosis (8) can be precipitated by side effects of medication (anticoagulants, antiepileptic drugs, antacids, corticosteroids), metabolic disorders, a number of illnesses (chronic pancreatitis, malnutrition syndrome, rheumatoid arthritis, cancer) and endocrine disorders (increased or decreased secretion of parathormone and thyroid hormones, decreased secretion of estrogenic hormones, the disorder in type 1 diabetes and 2).

The importance of senile osteoporosis as a form of primary osteoporosis is increasing. With aging, the concentration of biologically active parathormone (PTH, 1-84) increases. The main physiological regulatory mechanism of synthesis and release of parathormone is under a negative feedback of calcium ion concentration and vitamin D in plasma (9). An increase of PTH concentration causes a rapid increase in bone re-absorption, due to the increase in number and activity of osteoclasts. This consequently increases the mobilization of calcium and phosphate from bone and bone demineralization (10). The increased PTH levels in our group of women were probably caused by vitamin D insuficiency. However, 25(OH)D3 was not measured in this study.

Our results are in accordance with other studies, confirming the negative effect of thyroid dysfunction on bone metabolism. The function of the thyroid gland affects bone remodeling. Our study has shown that 69% of participants had normal thyroid function, but that 17% had hyperthyroidism and 14% hypothyroidism (Table 1). Thyroid hormones, in physiological concentrations, contribute to building bones, stimulating osteoblast activities. However, in patients with hyperthyroidism, there is low osteoid bone matrix formation. Hyperthyroidism in the elderly causes rapid bone re-absorption (11). The risk of femoral neck fracture increases. Osteoid matrix strength is in direct correlation with TSH suppression (12). In hypothyroidism there is a reduced number, activity and life span of osteoblasts, secretion of calcitonin, which increases the secretion of PTH levels resulting in degradation of bone, osteopenia or osteoporosis.

Conclusion

Thyroid and parathyroid dysfunction should be diagnosed in women will senile osteoporosis, in order to improve therapeutic intervention.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Hormonal and surgical characteristics of infertile patients with endometriosis

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Background

Current findings describe a multifactorial mechanism of endometriosis-associated infertility, suggesting that among others, ovarian endometriotic cysts, inflammatory changes, hormonal abnormalities and hyperprolactinemia might interfere with reproduction and the amount of functional ovarian tissue.

Aim of the study

In a retrospective study, 209 women receiving reproductive surgical intervention were included. These were subdivided as follows: (i) infertile women with moderate to severe endometriosis (rAFS III-IV), (ii) minimal to mild endometriosis (rAFS I-II), and (iii) without endometriosis. Serum levels ofanti-Müllerian hormone (AMH), Sex hormone-binding globulin (SHBG), Dehydroepiandrosterone sulfate (DHEAS), Thyroid-stimulating hormone (TSH), prolactin and fibrinogen were evaluated, as well as anatomical findings.

Results

An association between the severity of endometriosis and blocked Fallopian tubes was observed (p=0.016). AMH, DHEAS and fibrinogen were significantly different within the mild and severe endometriosis group (2.53±1.36 ng/ml vs. 0.68±0.44 ng/ml, p<0.001; 1.86±0.65 µg/ml vs. 1.55±0.69 µg/ml, p=0.026; 311.00±74.05 mg/dl vs. 379±326 mg/dl, p=0.025, respectively). No associations between serum prolactin levels and severity of endometriosis could be observed. Significantly lower AMH levels could be observed in patients with severe endometriosis compared to the control group (2.40±1.59 ng/ml vs. 1.09±1.23 ng/ml, p=0.001).

Conclusions

A significant decline in AMH levels can be observed in patients with moderate to severe endometriosis. Deep ovarian endometriosis seems to be associated with a decrease in the follicular ovarian reserve.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Whole slide characterization of lymphatic vasculature through cervical cancer progression: assessment of parameters with potential impact on tumoral dissemination

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Introduction

Lymph node extension is the main prognostic factor for overall and disease free survival in cervical cancer. In this context, lymphangiogenesis, formation of lymphatic vessels, has gained a lot of interest. In our study, we have developed a new original method based on computerized image analysis of lymphatic vessels on whole slide scanned tissue sections. Based on this imaging system, we have performed a detail characterization of tumoral lymphatic vasculature through cancer progression and correlated with a risk of nodal extension.

Methods

Seventy-nine cases of cervical neoplasia (12 CIN3, 10 FIGO stage 1A1 and 57 FIGO stage 1A2 to 1B1) and 10 cases of normal tissues were reacted with anti-podoplanin antibody (D2-40), a lymphatic endothelial marker. Immunostained structures were automatically detected on the whole slide. Whole field of the cervical tissue was assessed from the normal squamous tissue to transformation zone (TZ) and normal glandular tissue. An objective lymphatic vessel density (LVD), size and spatial distributions through cancer progression were assessed.

Results

In normal cervix tissue, prominent LVD was already detected under the TZ. During cancer progression, proportion of lymphatic vessels near the tumor cells (0.5mm) was increased. In invasive lesions, LVD was not correlated to lymph metastases but peritumoral LVD was associated with lymphatic vascular space invasion by tumor cells.

Conclusion

Whole slide analysis of LVD and vessel distributions is a robust objective method that is not subjected to interobserver variability as is the case with conventional optical microscopy. For the first time we provide evidence that TZ presents a specific lymphatic vessel distribution and density before the appearance of neoplastic events. Increased LVD in the closed peritumoral area promotes lymphatic vessel invasion by tumor cells.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Premenstrual disorders in female medical students: a cross sectional study

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Background

Many women of reproductive age experience some emotional, behavioral or physical symptoms in the 1-2 weeks prior to menses. Data from previous studies, conducted in different countries, show that premenstrual syndrome (PMS) is one of the increasingly common health problems of female students, but for the majority of students premenstrual symptoms are mild (3-8).
Premenstrual dysphoric disorder (PMDD) in women of reproductive age is frequently associated with limitations on social, academic, sports and daily activities. However, there is insufficient data on the PMDD prevalence and clinical features in medical students.

Objectives

To study the prevalence and clinical features of premenstrual disorders in medical students of the 1st-6th years of education.

Material and methods

This cross sectional study was performed in 541 female 1st-6th year medical students of ëhita State Medical Academy by questionnaire survey. The survey included the American College of Obstetrics and Gynecology (ACOG) criteria to diagnose PMS (2) and PMDD criteria according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (1). This study was approved by the Ethical Committee of the ëhita State Medical Academy (Chita, Russia). The data obtained were analyzed by χ-square test or z-criteria. The level of significant differences was evaluated at 5%.

Results

PMS was diagnosed in 21.2% of all interviewed students (115/541) and was estimated in 53.9% of them (62/115) as mild, in 40.9% (47/115) - as moderate and in 5.2% (6/115) - as severe.
PMS symptoms prevalence was significantly higher in elder students compare to the 1st-2nd year ones: it was diagnosed only in 4.52% of the 1-st year students; in 10.96% of the 2-nd year students (p_{1-2} <0,05) and in 12.43% of the 3-rd year students (p_{1-3} <0,01; p_{2-3} >0,05). Frequency of PMS in 4th-6th year students was the following: 15.29% in the 4-th year students (p_{1-4} <0,01); 17.02% in the 5-th year students (p_{1-5} <0,01; p_{2-5} <0,05) and 16.30% - in the 6-th year ones (p_{1-6} <0,01; p_{2-6} <0,05) (Fig. 1).
The symptoms of edematous form of premenstrual syndrome (with mastodynia, weight gain, abdominal bloating) was significantly more frequent in medical students of the 2nd year of education compare to others (Fig. 2). Emotional and psychical PMS symptoms (anxiety, stress-discomfort, nervous-anger, mild depression) and cephalgia were statistically more evident in the 4-6-year female medical students (Figs. 3-4).
Premenstrual dysphoric disorder (PMDD) symptoms were observed significantly rarely in the 1st-3d year students (p_{1-2} <0,05) (Fig. 5). The main PMDD symptoms were the following: severe emotional lability (77.8%), appetite disorders, overeating and predilection for special foods (70,3%), somnolence and fatigability (63%) and attention-deficit disorders (59.3%).

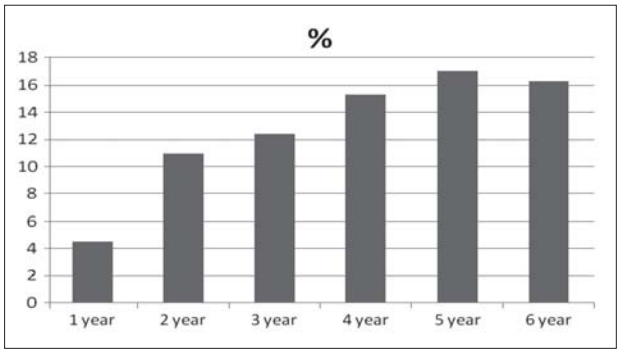

Fig. 1 - Premenstrual syndrome prevalence in medical students of 1st-6th years of education.

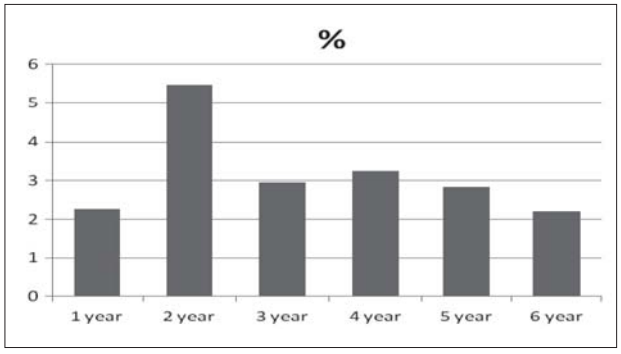

Fig. 2 - The frequency of edematous form of PMS in medical students of 1st-6th years of education.

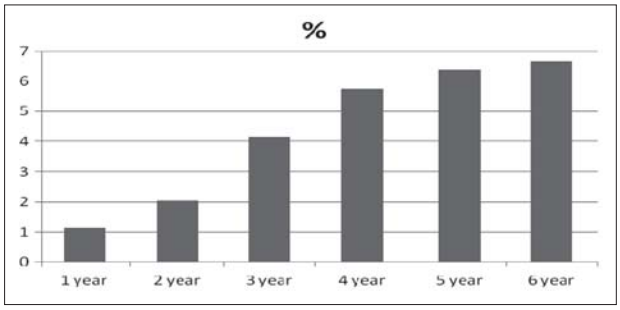

Fig. 3 - The frequency of psychical and emotional symptoms of PMS in medical students of 1st-6th years of education.

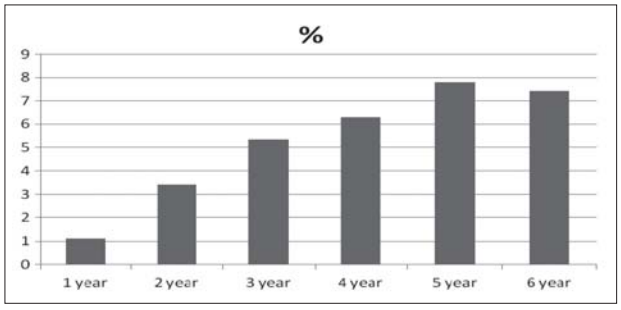

Fig. 4 - The frequency of cephalalgic form of PMS in medical students of 1st-6th years of education.

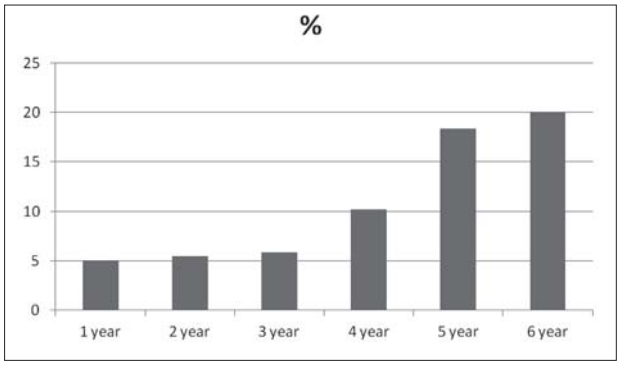

Fig. 5 - The frequency of PMDD in medical students of 1st-6th years of education.

Such symptoms as anxiety and tenderness, insomnia, anger and impatience were occur more rarely (in 22.2%, 18.5% and 14.8%, accordingly).

Conclusion

The results of our study show the significant frequency of premenstrual syndrome and premenstrual dysphoric disorder in medical students. The symptoms of edematous form of premenstrual syndrome are more frequent in 2nd year medical students, the prevalence of psychical and emotional PMS symptoms, cephalgia and PMDD is significantly higher in students of the 4th-6th years of education than in the 1st-3d year medical students.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Antenatal corticosteroids in women at risk for preterm delivery: an evidence update

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Preterm birth is an important cause of infant morbidity and mortality. Infants born preterm are at high risk of many complications: respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), periventricular leukomalacia (PVL), retinopathy of prematurity (ROP), patent ductus arteriosus (PDA), sepsis. RDS is the principal cause of neonatal mortality and morbidity (1). Liggins' incidental finding of the beneficial effect of antenatal corticosteroids (ACS) for promotion of fetal maturity is one of the most important discoveries in obstetrics (2,3). However, prevention of preterm birth is still insufficient and preterm birth rate continues to rise partially because of an increase in multiple gestations secondary to assisted reproductive technologies and an increase in maternal age at birth (1,2).

Two regimens of ACS are effective in the promotion of fetal maturity. Betamathasone is administered as 2 doses of 12 mg intramuscularly, 24 hours apart. Dexamethasone is administered as 4 doses of 6 mg intramuscularly, 12 hours apart. A number of studies have attempted to determine the superior agent and betamethasone has been described by experts as the preferable agent for treatment of women at high risk for preterm delivery (4). In fact, it was associated with a reduced risk for neonatal death and, in some studies, neonates exposed to dexamethasone had a higher rate of PVL (5-8). The result of the only randomized controlled trial comparing the 2 corticosteroids was recently published (9). No significant differences in rates of RDS, PVL, NEC, sepsis, or neonatal mortality were found. However, neonates exposed to betamethasone had a significantly higher rate of IVH (9). At this time, according to ACOG guidelines, both drugs are acceptable agents for promotion of fetal maturity.

Administration of a single course (SC) of ACS should be mandatory in the management of preterm delivery. In fact, it does not increase maternal morbidity and mortality and it is associated with an overall reduction in neonatal death, RDS, IVH, NEC, infectious morbidity, need for respiratory support, duration and cost of neonatal care (10).

However, the decrease of neonatal morbidity and mortality has been established only for infants born between 24 hours and 7 days after treatment. There is much controversy about how long the therapy with ACS should continue, and whether multiple courses (MC) should be administered if the women remain at high risk of preterm delivery 7 days or more after initial treatment.

Despite the lack of data confirming the safety and efficacy of additional exposures, administering MC of ACS became very common across the world in the early 1990s and the National Institutes of Health in 2000 stated that this kind of treatment should be firmly reserved to women participating in RCTs (11).

Recently, Crowther and Harding concluded that MC of ACS might reduce the occurrence and severity of neonatal lung disease and the risk of serious health problems in the first few weeks of life. However, these benefits were associated with a significant reduction of weight and head circumference at birth, and there was still insufficient evidence on the longer-term benefits and risks to recommend this treatment (12).

Moreover, a review and meta-analysis about benefits and risk of MC of ACS concluded that administration of MC of ACS does not add further benefits in term of composite neonatal morbidity, even if it is associated with some short-term benefits, as a decreased risk of RDS, of PDA, of use of surfactant, of ventilation support, and of any maternal side effects (13).

Peltoniemi et al wrote that weekly or beweekly repeated betamethasone might raise concern about long term consequences on neurodevelopment and metabolism (14).
The latest Cochrane review about MC of ACS states that the current available evidence shows no significant harm in early childhood, although no benefit (15). Further research is needed on the long-term benefits and risks for the women and babies.
Long-term results after administration of MC of ACS were recently published. For Wapner et al, at two years of age, there was no difference in weight, head circumference, or the Bayley Mental Development Index (MDI) and the Bayley Psychomotor Development Index (PDI) between infants exposed to SC versus MC of ACS (16). Although not statistically significant, the rate of cerebral palsy in infants exposed to MC, especially more than 4 courses, was found to be of concern and exposure to MC of ACS should be limited (16). Crowther et al, as well, reported a 2 years follow-up evaluation. They concluded that administration of MC of ACS reduced neonatal morbidity without changing either survival free of major neurosensory disability or body size (17). For Asztalos et al MC of ACS, given every 14 days, did not increase or decrease the risk of death or neurologic impairment at 18 to 24 months of age, compared with a SC of prenatal corticosteroid therapy (18). Continued follow-up monitoring of these children is necessary.
One strategy that has come into clinical use, without supporting data, is to administer a "rescue" steroid course (2).
Unfortunately, with all the benefits steroids provide to the fetus, we should never forget their effects on the mother. For most, there is little effect, but for a few, serious consequences can arise (19).
Steroid administration has been associated to maternal infection (20,21). However, risks for maternal infections (either antenatal or postdelivery) are either nonexistent, or only slightly increased (20,21). A well-established observation associated with steroid administration is some degree of maternal hyperglycemia (19). Risks for diabetic ketoacidosis after steroid administration are well known for known diabetic patients, but diabetic ketoacidosis has also been described in patients with no prior history of glucose intolerance (22). Evaluations of serum glucose and urine ketones after steroid administration would seem prudent and to maintain tight glucose control, an insulin drip may be necessary. Transient hypokalemia can occur after administration of steroids. If the steroids are given in conjunction with a beta agonist, risks for hypokalemia may be further increased (23). Hypokalemic paralysis has been described for patients with certain medical conditions, such as a variety of renal diseases, as well as hyperthyroidism (23,24). In conclusion when administering steroids, insulin dosages must be adjusted for the diabetic mother. A complete neurological examination, as well as routine glucose and potassium testing, should be considered, to determine whether additional precautions or treatments may be necessary for all patients who are prescribed steroids in pregnancy (19). However, in a international multicentre RCT, MC of ACS (every 14 days) were not associated with maternal side effects (25).
Future research should address those areas in which other studies have suggested problems, as length of drug effect, need for and risks of retreatment, ideal drug and dosage, maternal side effects and long-term effects. For the moment, obstetricians should be prudent in prescribing MC of ACS in women at risk of preterm birth.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Sexual health, reproductive and endocriniologic concerns in women after cancer: early findings from a multidisciplinary clinic

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The Odette Cancer Centre established an inter-professional clinic to address the sexual health, reproductive and endocrinology concerns (SHARE clinic) of women who had been treated for cancer. Initially focusing on women with gynecologic cancers, the clinic has expanded to offer services to women with pelvic cancers and more recently to young women with breast cancer. The clinic design had anticipated a high need for sexual therapy, instead we found a very high degree of menopausal symptoms and vaginal symptoms; symptoms that are very amenable to brief interventions by individuals experienced in menopause management.

This abstract reports on experience with the first 142 patients assessed through this clinic.

Patients were referred to SHARE Clinic by their oncologist, with referrals coming from gynecologic oncology or general surgery, radiation and medical oncologists. The majority of patients seen were referred with a diagnosis of gynecologic or breast malignancy: 11% ovarian, 48% cervix, 34% endometrium, 15% breast,all others 14 (10%). The mean age was 47. The proportion of women who had undergone pelvic irradiation varied by site

Across all sites of cancer, patients reported decreased interest in close physical contact (58.3%) and low or no desire (62.2%).

Vaginal complaints were extremely common. 75% reported vaginal dryness, 66% reported the vagina to be "smaller". 25% of women were not having intercourse. An additional 34% reported that they almost never experienced vaginal lubrication, and fully 75% experienced pain with intercourse.

The majority of vaginal symptoms could be easily managed with local estrogen. In a few cases lichen sclerosus was detected, in one case a prolapsed fallopian tube, and in one case a neuroma. Symptoms were more difficult to manage in women with breast cancer on aromatase inhibitors, in whom even local estrogen is avoided.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Assessment of hirsutism and associated factors in Brazilian women: necessity of definition of local standards

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Introduction

Hirsutism is present in 5-10% of women in reproductive age and polycystic ovary syndrome (PCOS) is the most common cause of it. It is defined as the presence of androgenic hair in hormonal dependent areas of the female body. Modified Ferriman-Gallwey score (mFG) is used to assess it and classically, hirsutism is considered when mFG is ≥8. Although largely used, that cut off sometimes is not adequate depending of the population assisted. In this study we investigated the relationship between mFG score, complaint of hair excess, clinical and laboratorial parameters in PCOS women.

Objectives

To assess the complaint of hair excess in PCOS women with mFG <8 and to study the relationship between the degree of hirsutism and clinical and laboratorial parameters.

Methods

A total of 299 PCOS women diagnosed with Rotterdam Criteria. Hirsutism was assessed through mFG score and classified as absent (<8), mild (8-12), moderate (13-18) and severe (≥19).
Clinical measurements: abdominal circumference and body mass index. Laboratory: total and free testosterone, HOMA-IR index, and basal insulin.
Statistical tests used were *Chi* Square, ANOVA, Bonferroni and Dunn's. In 77 subjects with mFG <8 complaint of hair excess was evaluated and in 245 patients, association between hirsutism score and clinical and laboratorial parameters were studied. Women with *Diabetes Mellitus* and impaired glucose tolerance were excluded from this analysis.

Results

Mean age was 25.4±5.3 (14-41 years). All PCOS women with score 7 and 50% of those with score 6 presented complaint of hair excess (Table 1). HOMA-IR index and basal insulin were positively associated with higher degrees of hirsutism (p=0.007 and p=0.015, respectively) (Table 2). BMI and AC presented no relationship with hirsutism degree but HOMA-IR and basal serum insulin did (Table 3).

TABLE 1 - RELATIONSHIP BETWEEN COMPLAIN OF HAIR EXCESS AND MODIFIED FERRIMAN-GALLWEY SCORE (MFG).

Complaint (n/%)	**Score 0** (n=11)	**Score 1-2** (n=12)	**Score 3-4** (n=15)	**Score 5** (n=10)	**Score 6** (n=12)	**Score 7** (n=17)
Yes	2 (18,2)	2 (16,7)	6 (40,0)	4 (40,0)	6 (50,0)	17 (100)
No	9 (81,8)	10 (83,3)	9 (60,0)	6 (60,0)	6 (50,0)	0 (0,0)

TABLE 2 - THE RELATIONSHIP BETWEEN mFG SCORE AND FREE TESTOSTERONE (T), HOMA–IR INDEX AND BASAL INSULIN (I0).

mFG score	Absent (score <8) (n=59)	Mild (score 8-12) (n=70)	Moderate (score 13-18) (n=81)	Severe (score ≥19) (n=35)	p
Free T ↑	34 (57,6)	43 (61,4)	53 (65,4)	25 (71,4)	0,557
HOMA > 2,7	19/57 (33,3)	22/60 (36,7)	47/79 (59,5)	18/34 (52,9)	0,007
I0 > 20	8/58 (13,8)	7/60 (11,7)	24/79 (30,4)	10/34 (29,4)	0,015

TABLE 3 - COMPARISON BETWEEN ABDOMINAL CIRCUMFERENCE (AC), BODY MASS INDEX (BMI), FREE TESTOSTERONE (T), HOMA-IR ÍNDEX, BASAL INSULIN (I0) AND mFG SCORE.

Modified Ferriman-Gallwey score	Absent (score <8) (n=59)	Mild (score 8-12) (n=70)	Moderate (score 13-18) (n=81)	Severe (score ≥19) (n=35)	p (ANOVA)
BMI (kg/m^2)	27,2±6,7[a]	28,1±7,5	30,7±6,3[a]	29,8±6,5	0,015
AC (cm)	84,9±14,2[b]	88,0±17,5	94,1±13,4[b]	93,5±16,9	0,008
Free T (pmol/mL)	55,9±33,1	56,5±34,6	69,6±42,4	68,9±39,1	0,061
HOMA*	2,75±2,48[c]	2,57±1,88[c]	3,95±3,29[d]	4,33±3,61[d]	0,001
I0* (mUI/mL)	12,7±10,6[d]	11,8±8,3[d]	18,4±14,6[e]	20,0±16,5[e]	<0,001

Bonferroni : a (p=0,018); b (p=0,014)
*Multiple comparisons of Dunn: all results were statistically significant except c (p=0,891), d (p=0,796) and e (p=0,959).

Conclusion

In the Brazilian population studied, an important number of women with mFG <8 have complaint of hair excess. BMI, abdominal circumference, free testosterone and HOMA-IR were not correlated to hirsutism. Women with score ≥13 presents greater HOMA-IR and basal insulin, which might lead to an increase of cardiovascular risk due. We suggest that the hirsutism score cut off in Brazil should be ≥6. Local standards of hirsutism score should be adopted to better treat women with androgen excess.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Visceral fat trough its products, a risk factor for endometrial cancer

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Introduction

Endometrial cancer is the sixth most frequent (4.9%) of all cancers diagnosed in the female population, the risk of endometrial cancer being higher in patients with high serum estrogen levels and/or low progesterone levels (1). Obesity increases systemic exposure to free estrogens via the aromatization of androgens in visceral adipose tissue, via the reduction of sex hormone binding globulin production, via the reduction of serum progesterone by anovulation, via the proinflammatory state (2).
Starting from the idea that in modern society the prevalence of obesity is increasing and that adipose tissue is directly correlated with a number of disorders, today we speak of *adipose tissue dysfunction* as an individual pathological condition. Adipose tissue is no longer considered just an energy storage organ, but a real endocrine organ whose hormones have not been completely characterized. Adipocyte biomolecules (leptin, adiponectin) have been identified, which are involved in the transmission of messages from adipose tissue to other target tissues.
Given that obesity and visceral obesity in particular is a risk factor for endometrial cancer, and that visceral adipose tissue secretes more than 50 inflammatory cytokines, in this study we aimed to assess the correlation between the plasma levels of inflammatory cytokines secreted by adipose tissue (leptin and adiponectin) and endometrial cancer.
We initiated a study that aimed to evaluate the presence of a correlation between intraabdominal adiposity assessed by ultrasounds (US) and endometrial cancer, as well as the presence of a correlation between intraperitoneal fat and cytokines (adiponectin and leptin) secreted by adipocytes in patients diagnosed with endometrial cancer.

Materials and methods

The study is a case-control analysis that includes 2 groups of patients: *group I* – 44 patients diagnosed with endometrial cancer, *group II* – 44 patients without gynecological pathology or inflammatory disorders (control group).
The diagnosis of endometrial cancer was made after a histopathological examination that analyzed the tissue material obtained following endometrial biopsy. Endometrial biopsy was performed in the case of considerable metrorrhagia, in the case of metrorrhagia during climax, as well as in the case of some aspects detected by US (endometrial thickness and vascularization).

Group I
Inclusion criteria
- Diagnosis of endometrial cancer following histopathological examination.
- Age 40-85 years.
- Willingness and ability to submit to the research procedures.

Exclusion criteria
- Other genital diseases than endometrial cancer.
- Treatment with systemic corticosteroids.
- Metabolic or endocrine diseases.
- Chronic autoimmune diseases.
- Malignant tumors.

Group II

Inclusion criteria

– Age 40-85 years.
– Willingness and ability to submit to the research procedures.

Exclusion criteria

– Other genital diseases than endometrial cancer.
– Treatment with systemic corticosteroids.
– Metabolic or endocrine diseases.
– Chronic autoimmune diseases.
– Malignant tumors.

After the performance of clinical examination and anthropometric measurements: body mass index (BMI), abdominal circumference (AC), these patients underwent US examination that determined intraperitoneal fat.

BMI was calculated by the formula BMI = weight (kg)/[height (min)]^2. AC (cm) was measured in orthostatism, at umbilical level. Ultrasonographic exploration (Voluson 739) was performed in dorsal decubitus at the end of a normal expiration, after a 12 hour digestive rest, in order to evaluate visceral fat deposit. The visceral fat area determined by ultrasound was calculated using the formula 9.008+1.191×[distance between the inner side of the right abdominal muscle and the splenic vein (mm)]+0.987×[distance between the inner side of the right abdominal muscle and the posterior wall of the aorta (mm)]+3.644×[fat thickness in the posterior wall of the right kidney (mm)] (3).

From each subject included in the study, 6 ml fasting blood were taken by venous puncture and collected in test tubes without anticoagulant for the measurement of plasma leptin and adiponectin levels. The serum obtained by centrifugation was divided and stored in 600 µl freezing tubes at a temperature of –30°C in order to avoid repeated freezing-thawing cycles. The serum leptin concentration was determined by the sandwich ELISA technique using the Human Leptin Imunnoassay DLP00 kit, R&D Systems USA. The serum adiponectin concentration was determined by the sandwich ELISA technique using the Human Total Adiponectin/Acrp30 Immunoassay DRP300 kit, R&D Systems USA.

All parameters were included in the study database, and version 13 of the SPSS software and Microsoft Excel with Analysis Tool Pack were used for statistical analysis. The Kolmogorov Smirnov test was applied for the testing of normal distribution. The Student t test was used for the comparison of the means and the Mann-Whitney test for rank comparison in two independent samples.

The informed consent of all patients was obtained.

Results

In the control group the mean visceral fat area was 142.41±23.06 cm^2 (with limits between 93.15 and 197.24), while in the group of patients with endometrial cancer the mean visceral fat area was 252.72±57.14 cm^2 (with limits between 141.56 and 361.83 cm^2). Thus, there was a statistically significant difference in intraperitoneal fat between the two groups ($p<0.0001$) (Fig. 1).

The plasma levels of the adipokines monitored in the study – leptin and adiponectin – are shown comparatively between the two groups in Table 1.

The plasma adiponectin level in the group with endometrial cancer is 7374.17±4701.35 ng/ml (with limits between 1739.95 and 20623.48 ng/ml), and in the control group 11045.68±4920.93 ng/ml (with limits between 2803.99 and 23494.22 ng/ml), with a

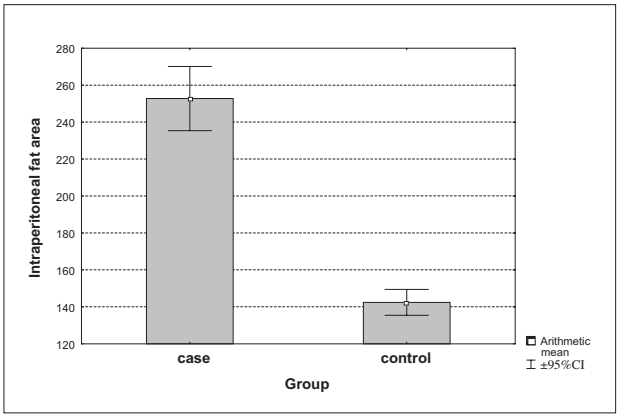

Fig. 1 - Comparison of intraperitoneal fat between the two groups.

TABLE 1 - COMPARISON OF PLASMA ADIPOKINE LEVELS BETWEEN THE TWO GROUPS.

		Arithmetic mean	Standard deviation	Standard error	95% confidence interval		Minimum	Maximum	p
Adiponectin ng/ml	Control	11045.68	4920.93	741.86	9549.58	12541.78	2803.99	23494.22	<0.00001
	Case	7374.17	4701.35	716.95	5927.30	8821.03	1739.95	20623.48	
Leptin pg/ml	Control	17103.79	12002.64	1809.47	13454.65	20752.92	2271.37	50335.20	<0.00001
	Case	40675.5	27912.73	4256.65	32085.23	49265.77	3151.76	122570.98	

statistically significant difference between the two groups. The plasma leptin level in the group with endometrial cancer is 40675.50±27912.73 pg/ml (with limits between 3151.76 and 122570.98 pg/ml), being significantly increased compared to the control group, where a value of 17103.79±12002.64 pg/ml (with limits between 2271.37 and 50335.20 pg/ml) is found.
The analysis of the relationship between intraperitoneal fat and plasma adipokine levels shows a positive linear relationship between intraperitoneal fat and the plasma leptin level (Fig. 2), while the plasma adiponectin level is in a negative linear correlation with intraperitoneal fat (Fig. 3). The correlation coefficient between intraperitoneal fat and leptin is r=0.43, p=0.00003 indicates a significant correlation between intraperitoneal fat and leptin; 25% ($d=r^2=0.25$ – diagram) of the leptin value is due to intraperitoneal fat. The correlation coefficient between intraperitoneal fat and adiponectin is r=-0.23, p=0.03 indicates a significant correlation between intraperitoneal fat and adiponectin; 5% ($d=r^2=0.05$ – diagram) of the adiponectin value is due to intraperitoneal fat.

Discussion

The risk of cancer disease in general increases with the increase in body weight, which is demonstrated in both men and women. In economically developed countries, endometrial cancer is associated with obesity in a proportion of 40% (4).
Regarding the visceral distribution of adipose tissue, intraabdominal obesity is considered a low-grade chronic proinflammatory state (5). In the context of endometrial cancer, visceral obesity as a risk factor is associated with a chronic inflammatory process, confirmed by the increase of inflammatory markers in the systemic circulation of obese patients (6). This study supports the idea that visceral adipose tissue is a risk factor for endometrial cancer; there is a statistically significant difference in intraperitoneal fat between the two groups.
Visceral adipose tissue has the capacity to secrete many substances that can be involved in the pathogenesis of endometrial cancer. Adiponectin is an adipocytokine exclusively secreted by adipose tissue. In women with increased plasma adiponectin levels, the risk to develop endometrial cancer is 50% lower compared to women with low adiponectin levels. This inverse correlation persists after the BMI correction, suggesting that adiponectin is a predictor for endometrial cancer, independently of obesity (7).
The association between low plasma adiponectin levels and endometrial cancer might be explained by the antiangiogenic properties of adiponectin. Adiponectin inhibits angiogenesis both in vivo and in vitro. Adiponectin inhibits tumor growth by suppressing the development of neoformation vessels in rats. These findings support the idea that low adiponectin levels are associated with increased angiogenesis required for the development of endometrial cancer (8). In this study, plasma adiponectin levels are significantly lower in the group of patients with endometrial cancer compared to the control group.
Inflammation and insulin resistance are important risk factors for endometrial cancer. Due to the interrelations between adiponectin and these risk factors, the implication of adiponectin in the etiopathogenesis of endometrial cancer is an increasingly supported hypothesis (9).
The evidence gathered so far shows that the measurement of adiponectin can be a useful screening method for the early detection of cancer correlated with obesity. Moreover, adiponectin or its homologues may be antioneoplastic agents and may have important therapeutic implications.

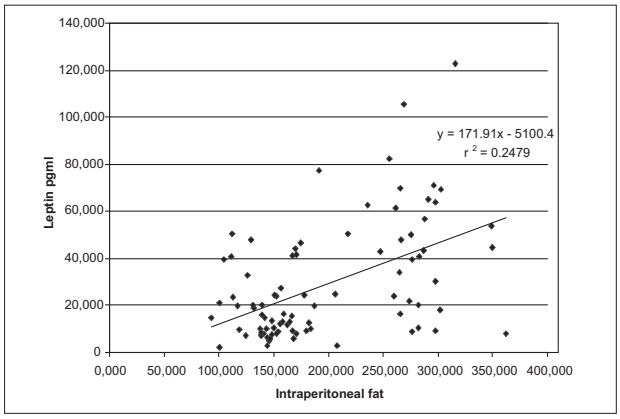

Fig. 2 - Correlation between leptin and intraperitoneal fat.

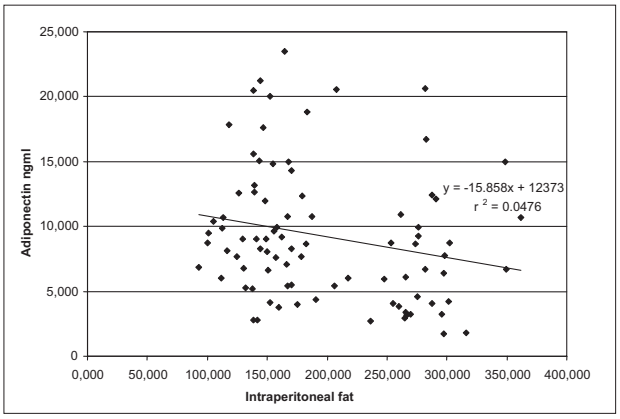

Fig. 3 - Correlation between adiponectin and intraperitoneal fat.

Regarding leptin, it was initially considered that this is only produced by adipose tissue, but subsequent in vivo and in vitro studies have identified other origins, the stomach, liver, placental and amniotic trophoblast cells (10,11). Regarding adipose tissue, the secretion of leptin occurs in various areas: subcutaneous, retroperitoneal, omental, perilymphatic (12).

Obese persons have high serum leptin levels, but this does not cause weight loss, suggesting that overweight is correlated with leptin resistance.

The decrease in serum leptin levels is correlated with the alteration of the balance between proinflammatory cytokines and antiinflammatory cytokines (IL-10, IL-1R) (13), supporting the hypothesis that leptin can affect antiinflammatory cytokine production through the activation of *signal transducer and activator of transcription 3* (STAT-3). Inflammatory cells can secrete leptin, which in its turn can intensify the inflammatory process (14). The induction of leptin by these inflammatory stimuli can be considered part of the systemic acute phase reaction (15). Proinflammatory cytokines such as TNF-α and IL-1β contribute to the increase in serum circulating leptin levels through the release of leptin stored in adipose tissue (16).

In contrast to increased serum leptin levels, low serum adiponectin levels are found in obese persons, which are inversely proportionally correlated with intraperitoneal fat in this study, as well as in other literature studies (17).

Abdominal adipose tissue through the mediation of adipocytes can change the plasma levels of adipokines (leptin and adiponectin) and secondarily, it can favor endometrial carcinogenesis.

Conclusions

1. Intraperitoneal fat is significantly higher in the group of patients with endometrial cancer.
2. Endometrial cancer is associated with low plasma adiponectin levels and high plasma leptin levels.
3. Visceral fat has a positive linear correlation with plasma leptin levels and a negative correlation with plasma adiponectin levels.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

PCOS - Should we screen for MetS and IR in women diagnosed with PCOS?

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Introduction

PCOS is one of the most common female endocrine disorders. It's a complex heterogenous disorder of uncertain etiology, though there is evidence that it can to a large degree be classified as a genetic disease (1,2,3).
PCOS produces symptoms in approximately 5-10% of women in reproductive age and it is thought to be one of the leading causes of female subfertility and the most frequent endocrine problem in young age (4,5,6,7).
The principal features are anovulation, resulting in irregular menstruation, amenorrhea, ovulation – related infertility and polycystic ovaries; excessive amounts or effects of androgenic hormones resulting in acne and hirsutism and insulin resistence, often associated with type 2 Diabetes and high cholesterol levels.
PCOS has been strongly associated with metabolic derailments such as MetS and IR. (Korhonen et al., 2001; Apridonidze et al., 2005; Dokras et al., 2005).
Both conditions imply changes in carbohydrate and lipid metabolism and a constellation of risk factors for development of cardiovascular disease. (Legro et al., 1999; Talbott et al., 2000; Grundy et al., 2004; Orio et al., 2004; Vryonidou et al., 2005; Ehrmann et al., 2006).
Both the above mentioned conditions and progression of undetected insulin resistence in PCOS women can lead to type 2 Diabetes, which itself is a very important risk factor for future cardiovascular disease in women (Lo et al. 2008).
This illustrates perfectly the need of an early detection of IR and subsequent application of preventive measures in PCOS in women.

The group of women diagnosed with PCOS has broadened considerably after the application of Rotterdam criteria.
In 2003 a consensus workshop sponsored by ESHRE / ASRM in Rotterdam indicates PCOS to be present if any 2 out of 3 of the following criteria are met; 1. oligoovulation and / or anovulation, 2. excess of androgen activity, 3. polycystic ovaries (by gynecologic ultrasound examination) (other entities, are excluded (9)).
PCOS has been known in literature with different names, such as polycystic ovaries disease, functional ovarian hyperandrogenism, Stein – Leventhal syndrome, ovarian hyperthecosis, and sclerocystic ovary syndrome.
This condition acquired its most used name due to common signs of ultrasound examination of multiple (poly) ovarian cysts. The "cysts" are actually immature follicles, not cysts.
Most of the patients with PCOS have IR and / or are obese. Their elevated insulin levels contribute to, or cause the abnormalities seen in the hypothalamic – pituitary – ovarian axis, which will lead afterword to PCOS. Hyperinsulinemia, increases GnRH pulse frequently. LH over FSH dominance increases ovarian androgen production (10), decreases follicular maturation and decreases SHBG; all these steps contribute to the development of PCOS.
But IR is a common finding among patients of normal weight, as well as overweight patients.
Under these circumstances, we need to figure if we should screen for the presence os MetS and IR in all patients diagnosed with PCOS and possibly find out what subgroups are more prone to develop these metabolic complications.

Materials and methods

Our study was conducted between May 2008 and December 2009.
It comprised females with PCOS, who's primer intention was to conceive. These patients were refered to our clinic for infertility problems.
All women presenting with oligomenorrhea (mean interval between bleedings >/=35 and <182 days) or amenorrhea (mean interval between bleedings >/=182 days) were systematically evaluated in our outpatient clinic.
Information was obtained regarding age, race, history of cycle abnormality, medical and family history, previous, or present use of medications, presence of acne and hirsutism, BMI, blood pressure, waist and hip circumference, fasting early morning endocrine profile, lipid, glucose and insulin.
A systematic pelvic ultrosonography was performed also in 3D images.
The diagnosis of PCOS was based on Rotterdam criteria, according to which, the diagnose of PCOS was considered positive, if at least 2 out of the following criteria were present: oligo / amenorrhea, clinical or biochemical hyperandrogenism and PCO in ultrasound examination.
Clinical hyperandrogensim was defined as the presence of hirsutism and / or acne, while biochemical hyperandrogenism was present if the calculated free androgen index (FAI = testosterone / sex hormone – binding globuline x 100) was >4.5 (Vermeulen et al. 1999).
PCO was defined as the presence of at least one ovary >10 cm3 in volume, and / or at least one ovary with 12 or more follicles measuring 2-9mm in diameter.
Other etiology, that could mimic PCOS like, Cushing syndrome, or androgen producing neoplasms ect, were not subject of this study, so were left out of it.
MetS was defined according to NCEP ATP III guidelines. MetS was considered a positive diagnose if at least 3 out of 5 features were present:

1. Waist circumference > 88 cm
2. Serum triglycerides >/= 1.70 mmol/l
3. HDL – cholesterol <1.30 mmol/l
4. Blood pressure >/= 130/85 mmHg
5. Fasting plasma glucose >/= 6.11mmol/l

IR was assessed using the HOMA (fasting insulin x fasting glucose/ 22.5).
A threshold point of 3.8 was used in this study as we have read before in Kauffman et all (2002).
A HOMA–IR value >3.8 probably reflects severe IR, because the threshold of 3.8 was based on inslune concentration above the upper limit of normal after a 100 g oral glucose tolerance test as standard test.
The entire group presenting oligo or amenorrhea, underwent the screening examination between May 2008 and December 2009.
The physical examination included blood pressure measured after a 10 min. rest, with a proper inflatable cuff size, adapted to the upper arm circumference in order to meet individual needs of each patient.
Waist circumference, was measured in the standing position halfway between the lower ribs and the superior anterior iliac spine of the pelvis.
The hip circumference was measured at the level of pubic symphysis.
Hirsutism was defined after the Ferriman – Galwary score (11).
The original score included 11 body areas to assess hair growth, but it decreased afterword to 9 body areas in the modified method:

1. Upper lip
2. Chin
3. Chest
4. Upper back
5. Lower back
6. Upper arms
7. Forearms (deleted in the modified method)
8. Thighs
9. Legs (deleted in the modified method)

Ultrasound examination was performed with a transvaginal transducer.

Results

Our study, had a modest group of women diagnosed with PCOS, as explained above, all of which were excluded from any PCOS mimic pathologies.
As a result of all the above mentioned examinations performed to all the women treated in our clinic, we found the following results, regarding the prevalence of the two most important endocrine disorders.
The prevalence of Mets and IR were respectively 24.9% and 24.3% (p, 0.001) and the prevalence between anovulatory women with or without hyperandrogenism was 23.1% and 13.9% (p 0.001)
The Mets prevalence also differed among women with or without polycystic ovary 23.1% and 63.8%.
The results, clearly show the significant difference in the prevalence of MetS between different phenotypes of PCOS.
What appeared to be an interesting finding was the high prevalence of both Mets and IR in the subgroup without any ultrasonographyc finding.

Conclusion

In conclusion, from our study we can afferm that the prevalence of MetS and IR varies between the phenotypic groups of PCOS. Specially hyperandrogenemia PCOS phenotypes resulted in higher risk for MetS and IR.
Though, it is still a concern, whether all women should from a clinical point of view be part of a screening process which has it economical cost.
As we have explained during this article, the group chosen is small and there is lack of materials to refer to, so further evaluation will be needed. For as much as we can say now, it Is our modest opinion to advise young women, in process of conception, to stick to the screening program. This will not only help to conceive, but most important it will help them stay healthy, avoid further complications due to metabolic imbalances.

Discution

The current study, has it's limitations.
Our group is a small group, besides this is the first time, a study of this kind was ever conducted in our country as far as we are concearned. So there is almos t no data, to wich we can refer, no references to help, therefore, it will need further evaluation.
Even in this moment, we are still collecting datas of women resulting with PCOS, hoping in a future re proposing this article with enriched datas.
Other limitations, include the establishment of IR. It is a very gradual phenomenon wich makes the threshold values artificial. Furthermore, patients with HOMA – IR <3.8, may still at some degree have IR.
In this study, as in most studies like-wise, the NCEP – ATP III was used for the assessment of Mets. Had we instead used the IDF definition of Mets its prevalence would have appeared to be much higher, almost double as high as it resulted from our study, vased on NCEP ATP II, because of stricter waist circumference criterion.
It is not yet known why women with PCOS tend to have a more android body fat distribution, which makes them prone to metabolic disturbances, leads to hyperandrogenism, which precludeas a vicios cycle of hyperandrogenism,hirsutism and metabolic abnormalities.
From a clinical point of view, it may be questioned weather all women should be screned for IR and Mets. It is our opinion, based on datas gathered in these 2 years of study, that screening for both conditions, to help women of very young age and fertile age, get the proper treatment in order to avoid future complication, is a wise approach.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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FSH-secreting hypophysis tumor - a new therapeutic option

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Introduction

Hyperprolactinemia is a condition characterized by excessive secretion of prolactin. Pituitary tumors are its most frequent causes, among them, the most common is prolactinoma. Other pituitary tumors may cause an increase in prolactin release by compressing the pituitary stalk and preventing dopamine which inhibits pituitary prolactin production, from reaching the pituitary. These other tumors may lead to a misdiagnosis of the etiology and treatment of hyperprolactinemia, due to other causes such as FSH-secreting pituitary tumors.

In women, FSH-secreting pituitary tumors may be diagnosed at any age. In the menacme, these tumors may cause amenorrhea and ovarian hyperstimulation with increased ovarian volume and formation of ovarian cysts.

FSH and estradiol (E2) levels may vary in tumors secreting FSH and this creates difficulty in diagnosing these tumors. Prolactin levels may also be elevated by pituitary stalk compression, as previously mentioned. Due to the variation in laboratory findings, immunohistochemistry of the tumor is important to confirm the diagnosis.

Surgical resection of FSH-secreting tumors is currently the first choice of treatment. Radiation therapy may be another option. Only a small number of studies have analyzed the use of GnRH antagonists in the treatment of these patients.

This study describes a case report of a patient with FSH-secreting pituitary adenoma manifesting Ovarian Hyperstimulation Syndrome.

Objectives

To draw attention to other non-prolactinoma pituitary tumors that may cause an increase in prolactin release by pituitary stalk compression and describe a case report of a FSH-secreting pituitary tumor managed with a new therapeutic option – Ganirelix, a GnRH antagonist.

Methods

To report a case of a 26-year old patient who received a diagnosis of a FSH-secreting pituitary tumor with Ovarian Hyperstimulation Syndrome followed at the Outpatient Facility of the Department of Obstetrics and Gynecology in the *Universidade Estadual de Campinas* School of Medicine.

The diagnosis was made by laboratory testing and imaging (computerized tomography-CT and magnetic resonance imaging-MRI of the sella turcica). The patient underwent pituitary surgery and during follow-up treatment she was given Ganirelix.

Results

The patient received follow-up care at the Outpatient Facility of the Department of Obstetrics and Gynecology in the *Universidade Estadual de Campinas*, due to secondary amenorrhea. During the investigation, laboratory tests were performed and hyperprolactinemia was found (prolactin 269ng/mL). Following this result, CT of the sella turcica was ordered showing a pituitary tumor measuring 17×12×10mm. Ultrasonog-

raphy (US) was also performed, revealing a increase in ovarian volume (right:320cm^3, left:513cm^3). This US finding was not initially taken into account and the patient received a diagnosis of macroadenoma of the pituitary. Cabergoline, a dopamine agonist, was used for the initial treatment of the patient.

Despite a fall in prolactin levels (to 2.86ng/mL) after treatment with Cabergoline was started, she remained in amenorrhea and began to complain of abdominal pain. MRI (Fig. 1) of the sella turcica was then ordered, showing a pituitary lesion measuring 25×15×18mm that invaded the cavernous sinus. After the performance of MRI, new laboratory studies were ordered, and high serum levels of E2 were detected (3637pg/mL), with normal levels of LH and FSH. Taken together, all these findings led us to the diagnostic hypothesis of an FSH-secreting tumor.

Partial resection of the tumor was performed by using the transsphenoidal approach. Diagnostic hypothesis was confirmed by histopathology that showed a chromophobic pituitary adenoma and immunohistochemistry that was FSH-positive in more than 50% of the adenoma cells, but negative for estrogen receptors (Fig. 2).

Following surgery, the patient experienced a period of transient decreases in E2 levels and ovarian volume. However, within 3 months there was an increase in ovarian volume, with abdominal pain and a new rise in E2 levels. Once again, MRI of sella turcica was performed, disclosing the persistence of pituitary tumor. A new therapeutic option was begun with the GnRH antagonist, Ganirelix, in combination with Cabergoline.

After 30 days of treatment, E2 levels were undetectable and the patient had a new reduction in ovarian volume (right:22cm^3, left:15cm^3). Unfortunately, Ganirelix had to be discontinued because it was no longer available for patient supply and only Cabergoline was maintained. There was a recurrence of elevated E2 levels and an increase in ovarian volume (right:89cm^3, left:31cm^3), with intense abdominal pain (Table 1). The performance of bilateral oophorectomy was required.

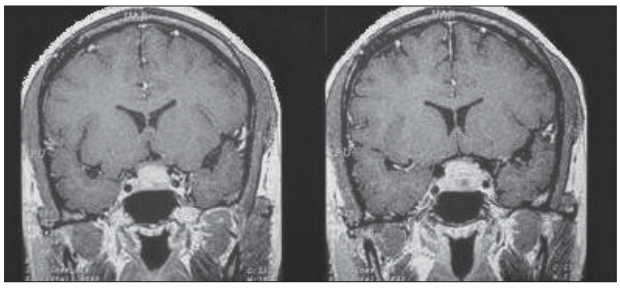

Fig. 1

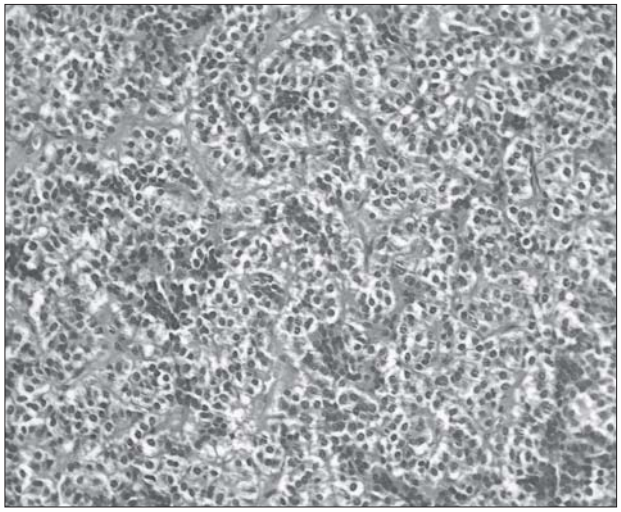

Fig. 2

Discussion

Pituitary adenomas that secrete FSH are rare. In women, they progress with amenorrhea with ovarian hyperstimulation. In this case report, the diagnosis of an FSH-secreting tumor was not made immediately because hyperprolactinemia associated with a tumor visualized initially by CT of sella turcica led to a misdiagnosis of pituitary Macroadenoma.

In FSH-secreting tumors, the serum levels of E2 vary widely. In the majority of the cases described, they are increased. Low E2 levels in some patients could be explained by extremely low LH levels, which is also important for the synthesis of E2.

Since FSH levels are variable and frequently normal, the reasons for ovarian hyperstimulation are debated. It is questioned whether there is an increase in FSH biological activity or a failure of the methodology used to measure this hormone. There could be a lack of detection of the active FSH components or FSH isomers produced by the tumor. The use of histopathology and immunohistochemistry are necessary to establish the diagnosis of FSH-secreting pituitary tumor.

A study using a suppression test with E2 suggested that FSH-secreting tumors do not respond to suppressing levels of circulating E2 as occurs with the normal pituitary gland. Thus, in the present case report the estrogen receptor was investigated by immunohistochemistry of the tumor tissue and was negative, indicating that high serum estrogen levels may not exert suppressive activity on tumor tissue due to the lack of receptors in these cells.

After 4 weeks of treatment using Cabergoline and Ganirelix, E2 levels were undetectable, ovarian volume decreased and the patient was asymptomatic. Unfortu-

TABLE 1 - HORMONE SERUM LEVELS AND OVARIAN VOLUME IN DIFFERENT MOMENTS: BEFORE SURGERY, AFTER SURGERY AND UNDER MEDICATION.

	Before surgery	35 days after surgery	100 days after surgery	Ganirelix and Cabergoline	Cabergoline alone, after Ganirelix was discontinued
FSH mIU/mL	13.0	11.7	8.1	7.6	8.8
LH mIU/mL	2.8	1.9	2.0	1.1	1.8
E2 pg/mL	3637.0	11.8	535.0	5.0	595.0
Ovarian volume right/left cm^3	320/513	–	34/63	22/15	89/31

nately, due to difficulty in drug acquisition, Ganirelix was discontinued. It was observed that Cabergoline alone was not capable of controlling ovarian hyperstimulation and there was a recurrence of clinical features and laboratory findings.

During medication use, a significant decrease in FSH levels was not identified. We believe that clinical improvement in relation to ovarian hyperstimulation may have occurred due to a reduction in LH levels and to a GnRH antagonist direct effect on granulosa cells – an inhibition on aromatase activity, in addition to its central pituitary action. This could contribute to a significant decrease in estrogen levels and improve the clinical condition of the patient.

Conclusions

Ganirelix, a GnRH antagonist is a therapeutic option that should be considered in patients with FSH-secreting pituitary tumors characterized by clinical findings of ovarian hyperstimulation. Despite the few studies on the subject, it seems to be an effective and non-invasive option, with a low morbidity, good tolerability and comfortable dosage regimen (1 dose per day).

Immunohistochemistry of the tumor should be performed not only to measure FSH levels, but also estrogen receptors. Immunohistochemistry would help understand the pathophysiology of these rare tumors and search for new therapies.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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A screening program for endometrial cancer in an asymptomatic postmenopausal population in our City: preliminary data

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Introduction

Endometrial carcinoma is the fourth most common type of cancer among women in Europe. In Italy it is the first female neoplasia, with more than 5000 newly diagnosed cases each year.
Targeted screening examinations for early detection, with endovaginal sonography followed by endometrial biopsy, seem reasonable for high-risk women (e.g., women with Lynch syndrome). Yet, even for these women up to now there is no real evidence for the benefits of this kind of screening.
Current scientific evidence does not support the screening of asymptomatic women. Uterine bleeding in a postmenopausal woman is the main sign of endometrial carcinoma. Pre- or perimenopausal women with acyclical bleeding should also undergo a thorough diagnostic evaluation, particularly if they present risk factors for endometrial carcinoma.
In postmenopausal patients an endometrial thickness of more than 5 mm is considered suspect. Hysteroscopy and fractionated uterine curettage are essential for a histological diagnosis.

Methods

Thanks to a financial support by the local administration of Tavagnacco, a small village near Udine, North-East Italy, we designed a special screening program with the aim of detecting endometrial cancer in asymptomatic postmenopausal women.
The first phase of the programme was to send a questionnaire to all women living in Tavagnacco and older than 48, in order to gain information about their health condition, pre- or post-menopausal status and reproductive history. The women also received an information leaflet with explanations about the screening program and an explicit request for participation. This phase was handled by the local administration, which transferred all the data to our clinic.
The second phase of the program was to invite for the screening all the women who had accepted to participate. For this purpose we organized an ambulatory once a week where all women underwent a thorough gynaecological visit and an ultrasound evaluation. Those women who showed a high endometrial thickness or were suspected to have a polyp, underwent a second level screening: a sonoisterography and an endometrial cytology.
We wish to point out that the data presented in this study are preliminary, because they are restricted to a part of our sample as the program is still going on; other data will be available in the future.
Inclusion criteria were: age, post-menopausal status, presence of the uterus and absence of bleeding.
Statistical analysis was performed by R (2.13.1 version) with a significativity of $p<0.05$.
The distribution of data was analyzed by Kolmogorov-Smirnoff test. Wilcoxon test, chi-square and Fisher test were used where necessary.
A multivariate logistic regression analysis was performed and ROC curves were created, to detect cut-off values for the description of the high-risk subgroup of patients.

Results

We enrolled 620 patients from August 2010 to September 2011: 429 women underwent the screening

program, 29 women were excluded because of a previous hysterectomy, 67 were not in a post-menopausal status and 95 women did not accept to continue the program.
Among 429 evaluated patients, 30 (7%) had a second level screening because they presented a high endometrial thickness or a focal image which suggested the presence of a polyp. Among these 30 patients 9 (2.1%) had an endometrial polyp, and only 1 of these (0.2%) was an endometrial cancer (adenocarcinoma T1aNxM0 G1).

Discussion

Endometrial cancer has an incidence of about 25 per 100.000 women. The overall 5 year survival is 80% and stage-related. There are two types of endometrial cancer: estrogen-related (type 1) and non estrogen-related (type 2). The likelihood of endometrial cancer can be assessed by ultrasound. Endometrial cancer is associated with a thickened endometrium as measured by transvaginal ultrasound. The histological diagnosis of endometrial cancer is usually made through endometrial sampling. Unfortunately, unlike type 1, an early type 2 endometrial cancer often causes only a subtle and focal thickening of the endometrium on a thin atrophic background, and it is more likely to be missed both by ultrasound examination and sampling. Current scientific literature therefore does not support mass screening for endometrial cancer.
Normally, ultrasound is not used for endometrial cancer screening in asymptomatic women. Patients often undergo a transvaginal ultrasound on the basis of indications which are unrelated to the endometrial status, e.g. with the purpose to study a pelvic mass or to understand the nature of pelvic pain. It is therefore very important to interpret correctly incidental ultrasound findings, such as thickened endometrium or the presence of a polyp. For instance, one has to understand when an endometrium in a post-menopausal woman with a good health condition is to be considered thickened. Studies conducted up to now involved women with bleeding, which means that the population considered for transvaginal ultrasound was symptomatic. These studies show that endometrial cancer is most unlikely in women with a thin endometrium. The proposed cut-off for endometrial thickness varies from 3 to 5 mm. Smith-Bindman et al. concluded that the risk of malignancy in a post-menopausal woman is 7.3% if her endometrial thickness is more than 5 mm, while it is 0.07% if this value is lower than the cut-off. More recently, Timmerman et al. proposed a cut-off value of 3 mm.
Our data confirm this finding and suggest that the best endometrial thickness cut-off to detect a cancer is 3.3 mm. However, the novelty of our study is the population considered, namely asymptomatic women. We actually tried to build up a mass screening involving all the post-menopausal women of Tavagnacco, women who did not experience an abnormal bleeding, in order to identify the best value for endometrial thickness.
Among the 620 patients recruited, 429 were able to do the screening program. In 9 women we detected a polyp, but only one of these was diagnosed as cancer. Polyps are often seen at ultrasound after menopause. The reported incidence varies between 13 and 17%. These polyps have a very low risk of malignancy: 0,1% as described in the study by Goldstein. Although our sample is more restricted than Goldstein's and our study is still going on, the data we have collected up to now seem to confirm the trend signalled in the literature.
Ultrasound examination is easy to perform and well tolerated, and it could therefore be an excellent screening technique for endometrial cancer in the general population. Evidence reported in the literature as well as the data presented by our study show an extremely low incidence of this cancer in asymptomatic populations. Hence, ultrasound is not feasible as a large scale screening because of its negative cost-benefit ratio. Actually, among all patients screened in our program, we only detected one case of cancer.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The lumbar DXA parameters and high risk fracture patients evaluated by Heel Quantitative Ultrasound. A study in 343 women

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Introduction

The Heel Quantitative Ultrasound (QUS) is a useful tool to fracture risk assessment. It uses mobile, non X-Ray, non-expensive devices. (1) Even the diagnosis of osteoporosis cannot be established by QUS as in cases evaluated by DXA (Dual Energy X-Ray Absorptiometry), the main concern in patients' management based on the fracture risk levels represent no more a problem since there are several studies using QUS parameters in order to evaluate the fracture risk. We used a combine parameter Stiffness Index (SI) in units (U) (applying some known cut offs) derived from QUS measurement to appreciated the women's risk of fracture. The correlation between DXA and QUS was intensely studied also the result must be stratified based on patient profile as age, previously osteoporotic fracture, secondary causes of bone loss. (2) There are several population studies related to the DXA QUS correlations, with different results that considerably improves if the clinical risk factors are also used. (3)

Aim

We analyze the lumbar DXA BMD (Bone Mineral Density) values into the high risk population according to the heel SI QUS levels.

Patients and methods

We included 343 women in menopause. The last menses were resumed at more than 45 years of age, regardless surgically or spontaneously. We enrolled the patients between January 2010 and November 2011. These were patients admitted in CI National Institute of Endocrinology from Bucharest, Romania. They were admitted for different diagnosis, not necessary bone metabolism diseases. The inclusion criteria was that the women never been previously treated for osteoporosis with antiresorbtives or anabolic agents. If the patients were treated with hormonal replacement therapy, we included them if they had at least one year since last month of therapy. We performed central DXA (lumbar spine) by a GE Lunar device, and heel GE Lunar Achilles QUS, which is considered the golden standard device for applying QUS. We used the cut – off of 54U for SI which marks the highest fracture risk group (SI ≤54U), or group 1. The patients with high risk of suffering a future low trauma fracture may be treated with antiresorbtives (as those diagnosed with osteoporosis by DXA or evaluated with increased 10-years probability of fracture by FRAX score) and the DXA is not necessary. According to some studies the SI ≥ 79U low means low fracture risk if the patient did not suffer before a fragility fracture (group 3). Based on serial observations, these patients do not need antiresorbtive therapy, only periodic follow up by QUS. (4) We considered the "medium fracture risk" group the women with SI between 54 and 79U (group 2). This analyze was performed mainly related to the DXA and QUS values, and less to the clinical risk factors profile as the presence of corticotherapy, diabetes mellitus or rheumatoid arthritis. The student ttest was used for statistical analyze (statistically significance was for $P<0.005$).

Results

Out of the 343 patients, 24 had high fracture risk profile (group 1), based on the SI levels. 176 patients were included in the medium risk group and 143 in group 3. The ranges of the SI values were between 42 and 54U. The ranges of the SI in group 3 were 79-135. The av. age was for the group 1 60.92±8.83 yrs, group 2 56.34±9.1 years, group 3 55.99±7.41 years. The high risk patients were statistically significant older than low risk (P<0.005). The av. BMD was 1±0.18 g/cm2 (group1), 0.98±0.16 g/cm2 (group2), 1.06±0.2 g/cm2 (group3). (Fig. 1) The student ttest between high risk and medium risk group was p=0.65. In contra to expected results, the av. BMD was higher in high fracture risk women compare to those with medium fracture risk but the results were not statistically significant. Generally, the patients with intermediary values of the SI QUS (related to the SI cut-offs we mentioned before) represent a heterogeneous category of patients because the management of these patients is related to the clinical risk factors profile. As expected, the values of the BMD between high and low fracture risk groups was statistically significant (p<0.005).

We also evaluated for each patient the Body Mass Index (BMI). The av. values of BMI for each group were: 27.62±7.93 kg/m^2 (group 1), 27.86±9.1 kg/m^2 (group 2), 29.93±5.46 kg/m^2 (group 3). The differences between the SI groups were not statistically significant: for high-low fracture risk groups p=0.07 (but with some correlation, meaning that the BMI is higher as the SI is higher), and between high-medium fracture risk groups p=0.85. The average value of the BMI is situated within the overweight area, while for low fracture risk patients is closer to obese ranges.

Based on WHO criteria, we appreciated the osteoporosis/osteopenia patients into each group starting from the BMD DXA values. (5) The repartition of the DXA groups in the high risk group was: 29% of the patients had osteoporosis, 54% had osteopenia and 17% had normal DXA. The low fracture risk patients had osteoporosis 6.29% of them, osteeopenia 42.65% of the women and normal DXA was found in 51.04% of the patients. The medium fracture risk group included 21.59% women with T-Score less than -2.5, 59.65% of patients with T-Score between -1 and -2.5 and 18.75% of women with normal T-Score. (Fig. 2) The percent of the postmenopausal women diagnosed with osteoporosis increases with SI groups (a lower SI associates a higher chance of osteoporosis). In high fracture risk group, more than a half of the patients have osteopenia. But the percent of patients with osteopenia according to DXA is almost similarly into the other two groups. We consider that this population is also heterogeneous, and the decision of treatment should be based in clinical risk factors phenotype or FRAX analyze.

Discussion

The QUS analyze is very useful in selected cases. The groups revealed by SI as high and low risk for fractures were statistically significant different based on lumbar spine BMD DXA. The percent of women diagnosed with osteoporosis is more than 4 times higher in patients with high risk compare to low risk but almost 50% of patients of each QUS fracture risk group have osteopenia. This raised the issue that in fact the risk of fracture judgment is important to be individual, in a complex equation when other risk factors as clinical

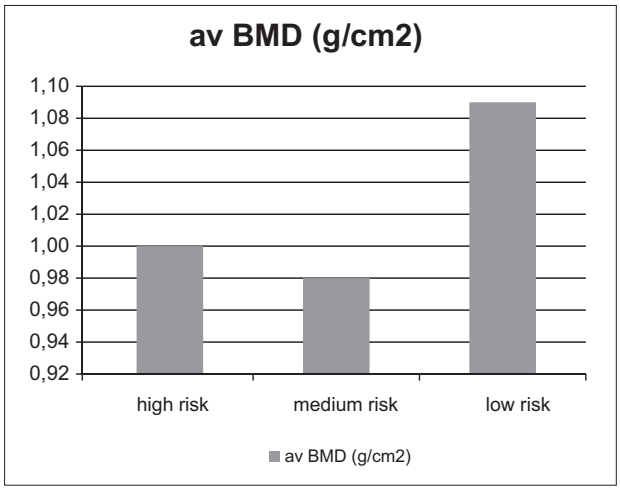

Fig. 1 - The average BMD (DXA) values of the three SI (QUS) based fragility fracture risk groups.

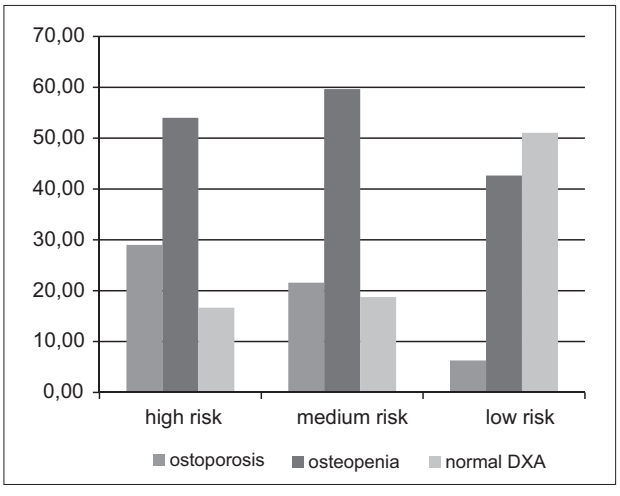

Fig. 2 - Percent of the patients with osteoporosis/osteopenia/normal DXA into each group of QUS fracture risk.

parameters have a role but this was not the case of our study. We conducted this analyze up from the idea that the risk groups for fracture as they are revealed by heel QUS should have some distinct features regarding the factors that play some roles in bone health as age or BMI (in an univariable analyze), and that the two most used tools of the bone evaluation as DXA and QUS may provide some interesting data.

Conclusions

The bone mass density is statistically significant lower in patients with high risk fracture based on QUS compare to low risk fracture patients. Less than a fifth of the patients with high risk fracture have normal DXA while most of them have osteopenia rather than osteoporosis. The study of the clinical risk factors is important also for a better fracture risk evaluation.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Influence of abdominal obesity on the calculated androgen parameters in the young women with different syndromes in Georgia

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Introduction

Hyperandrogenism refers to classical androgen-dependent signs, such as hirsutism, acne, alopecia. The frequent causes of hyperandrogenism are polycystic ovary syndrome (PCOS), nonclassic congenital adrenal hyperplasia (NCAH) and hyperprolactinemia. PCOS is a heterogeneous endocrine disorder that affects about 6-8% women worldwide (1). PCOS features include signs of hyperandrogenism, menstrual irregularities and infertility. NCAH is the result of 21α-hydroxylase deficiency. The symptoms of NCAH include menstrual dysfunction, hirsutism, acne and infertility (4). These syndromes clinically are very close to each other. Therefore, in a patient presenting with phenotypic features of PCOS, it is important to consider NCAH in the differential diagnosis. Hyperprolactinemia associated with amenorrhea, galactorrhea, infertility and mild hirsutism. Some clinical signs of hyperprolactinemia are similar to that of PCOS. In fact, the diagnosis of PCOS requires the exclusion of hyperprolactinemia (3). The aim of the study was to estimate correlations between abdominal obesity and calculated androgen parameters in patients with PCOS, NCAH and hyperprolactinemia.

Materials and methods

The study population consisted of 108 young women age 13-30 referred to our clinic for hirsutism, acne and/or irregular menstrual cycle. One of the main principles of our sampling was to choose women who were past at least 2 years after the menarche. They did not take any medication, including oral contraceptives, for at least previous 6 months. We had three groups. First group (n=40) was presented by patients with PCOS and fulfilled the diagnostic criteria for PCOS (Rotterdam 2003), group II (n=38) – by patients with NCAH diagnosed by high level of 17α-OHP and group III (n=30) - by patients with hyperprolactinemia. All of the patients underwent measurement of height, weight, waist and hip circumference, body mass index (BMI) (weight [kg] divided by height square [m2]) and the waist to hip ratio (WHR) as the ratio between the waist and the hip circumferences were calculated. Hirsutism was evaluated according to Ferriman - Gallwey scores in 9 sites of body. Total testosterone (TT), sex-hormone binding globulin (SHBG), free testosterone (FT), 17α-hydroxyprogesterone (17α-OHP) and prolactin (P) were measured with Enzyme-linked immunosorbent assay (ELISA). Calculations of free testosterone (cFT) and bioavailable testosterone (cBio-T) were performed using the formula available on the web site of the International Society for the Study of an Aging Male (ISSAM) (http://www.issam.ch/freetesto.htm). FAI was obtained as the quotient: 100 X (TT/SHBG) (5).
Statistical analyses were performed with SPSS software (statistical Package for the Social Sciences, version 17.0 for windows XP; SPSS, Inc, Chicago, I17). A p-value of less than 0.05 was considered to be statistically significant. Two-group comparison of continuous variables was performed using a two-sample *t*-test and Mann-Whitney U test. More than two group means were compared using the ANOVA with post hoc.

Results

First group was presented by 40 (37%) patients with PCOS, group II by 38 (35,2%) patients with NCAH

and group III by 30 (27,8%) patients with hyperprolactinemia. Hirsutism was detected in 87,5% patients with PCOS, in 71% - with NCAH and in 70% - with hyperprolactinemia. In patients with PCOS, NCAH and hyperprolactinemia were not detected any significant differences by hormonal and calculated androgen parameters.

There were high FAI levels in all patients groups. In PCOS women FAI mean was - 14,9; in NCAH women - 12,4 and in women with hyperprolactinemia – 12,9. A ratio of less than 8 of the FAI is considered normal in women. Hirsutism score did not differ between groups (Table 1). PCOS hirsute women had significant high level of cFT, than PCOS women without hirsutism. Abdominal obesity more frequently was detected in women with PCOS 20 (50%), than in women with NCAH -7(18,4%) and women with hyperprolactinemia -10 (33,3%). Higher body mass index (BMI) also was detected frequently in women with PCOS 17(42,5), than in women with NCAH – 7 (18,4%) and women with hyperprolactinemia - 11(36,7%).

TABLE 1 - COMPARISON OF CLINICAL, HORMONAL AND CALCULATED ANDROGEN PARAMETERS BETWEEN PCOS, NCAH AND HYPERPROLACTINEMIA PATIENTS GROUPS.

characteristics	PCOS	NCAH	Hyperprolactinemia	P-value
TT	2,4 (± 1,1)	2,4 (± 1,1)	2,2 (± 1,0)	p>0,05
FT	3,4 (± 1,9)	4,2 (± 2,8)	3,9 (± 2,5)	p>0,05
SHBG	28,3 (±21)	32,2 (± 26,4)	26,4 (± 21,8)	p>0,05
FAI	14,9 (± 12,4)	12,4 (± 9,4)	12,9 (± 8,6)	p>0,05
cFT	2,0 (± 0,8)	2,0 (± 0,7)	2,1 (± 0,8)	p>0,05
cBio-T	39,2(± 27,4)	38,9 (± 24,2)	42 (± 27,6)	p>0,05
Hirsutism score	13,8 (± 6,3)	13,5 (± 7,2)	14,5 (±8,4)	p>0,05

Note: Results are expressed as the mean ± SD.
*P value consider significant when it p<0,05. NS – not significance. Total Testosterone (TT); Free androgen index (FAI); free testosterone (FT); calculated free testosterone (cFT); calculated bioavailable testosterone (cBio-T), sex-hormone binding globulin (SHBG).

With regard to WHR, all patients were divided into two groups: 34,3% of women had abdominal obesity (WHR >0,80) and 65,7% of them had peripheral obesity (WHR<0,80). In patients with peripheral obesity hirsutism was revealed in 55 (77,5%) and in patients with abdominal obesity - in 28 (75,7%). There were small differences by calculated androgen parameters between groups of abdominal and peripheral obesity, but not significant. There was detected significant low level of SHBG in patients with abdominal obesity.

Discussion

In this study clinical, hormonal and calculated androgen parameters differences between the different androgen excess disorders were small, but FAI index was high in all syndromes. Carmina E. et al study had the similar evidences (2). According to this study, PCOS patients with hirsutism had significantly high level of cFT, than PCOS patients without hirsutism. Obesity is common in PCOS and affects between 30-70% of women. Our data confirmed that PCOS patients were more obese, than patients with other syndromes. As well as, in women with abdominal obesity SHBG concentrations significantly low, than in women with peripheral obesity. It is well known, that an increase of body weight and fat tissue is associated with several abnormalities of sex steroid balance. Such alterations involve both androgens and estrogens and their carrier protein, SHBG. In fact women with abdominal obesity usually have lower SHBG concentrations in comparison with women with peripheral obesity.

Conclusion

This study demonstrates that abdominal obesity was a common feature of PCOS. Hirsute women with PCOS had significant high level of cFT, than PCOS women without hirsutism. All women with abdominal obesity observed higher level of FAI, than women with peripheral obesity, but not significantly. SHBG level was significantly low in women with abdominal obesity. There were not any differences by hormonal and calculated androgen parameters between these syndromes.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Natural pregnancy prognosis for IVF/ICSI candidates using DuoFertility in comparison to other expectant management methods

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Introduction

High spontaneous pregnancy rates in couples with unexplained infertility have been demonstrated (1-3), which leads to the question of whether expensive and invasive fertility treatment should be the first choice for these couples. The pregnancy rate for couples with unexplained infertility using expectant management methods, compared to IVF and other ART methods, has been studied by several teams (4,5), but has not led to a consensus on the method of choice. Some studies indicate that the pregnancy rate using IVF is higher than with expectant management (5), while others show that there is no difference (6). When reviewing the evidence, the authors of the Cochrane review indicated that any effect of IVF relative to expectant management in terms of live-birth rates for couples with unexplained infertility remains unknown (7,8).

Current options for patients

GP based expectant management
Following the UK guidelines for the management of the infertile couple, couples diagnosed with unexplained infertility (1/3 of infertile couples) will be advised to "keep trying" for a third year prior to eligibility for assisted reproductive technologies. The natural pregnancy rate is estimated by NICE at 11% over this third year.

Specialist GP expectant management
In Ireland, a study indicated for that infertile couples GP using the Creighton Model fertility charting and optimally timed intercourse as well as medication when thought necessary lead to 35.5% pregnancy after 12 months. Note that although the study included some patients with infertility of at just one year duration, approximately one-third had previously undergone some form of ART. The most common medications given to patients included clomiphene citrate (75%) to stimulate ovulation, human chorionic gonadotropin (hCG) (67%) or progesterone (18%) in support of luteal hormonal production, and a range of medications to enhance cervical mucus production (71%) (9).

Fertility clinic expectant management programmes
There have been no publications of specific clinic led expectant management programmes in the UK; however in the Netherlands several studies have shown that this can offer comparable chances of pregnancy to IVF or IUI for couples with a good prognosis such as unexplained infertility. This was shown to result in a 65% spontaneous pregnancy rate for such couples over 5 years and the average time to pregnancy was 5.7 months (10).

Expectant management by Telemedicine
A recent study shows that patients using a new "telemedicine" approach to expectant management had a significantly higher chance of conception than using classic "expectant management" methods. The DuoFertility programme resulted in an average clinical pregnancy rate of 39% after 12 months of use, which compares favourably to the in-vitro fertilisation clinical pregnancy rate for couples suffering from unexplained infertility (31.8%) (11). Note that 3% of patients were taking clomifene citrate and none were taking other fertility medications at the time of conception, which is a significantly lower rate of drug intervention that appeared necessary in the Irish study to achieve similar outcomes. As with the pregnancy outcomes, concurrent interventions are patient reported.

Materials and methods

DuoFertility programme compared to the HFEA Register
When considering the impact of expectant management approaches on provision of assisted reproductive services, it is important to ensure good matching of study groups.
For detailed comparison of outcomes from expectant management and assisted reproduction, a retrospective cohort study of the first 500 couples on the DuoFertility programme is provided. Of the 500 couples, 226 met the criteria for IVF/ICSI in the UK and provided data sufficient for comparison to UK national statistics on in-vitro fertilisation (IVF). The most recent publicly available HFEA dataset (2008) was used for reference, excluding any use of donor embryos, eggs or sperm (as expectant management is based on natural conception), but including both IVF and ICSI cycles. Of 27,360 cycles recorded in the HFEA 2008 dataset, 3,460 were excluded as containing donor sperm, eggs or embryos. Of the remaining 23,900 cycles, 7903 (33%) were for unexplained infertility, 6554 (27%) were for female-only factors, 7746 (32%) were for male-only factors, and in 1697 cases (7%) underlying causes were identified for both partners. Although the HFEA Register does not differentiate mild identified causes of infertility (such as sperm counts of 5-20M/mL or a single blocked fallopian tube), anecdotal evidence suggests that more than a quarter of identified causes are mild.
The major determinants in pregnancy rate, both for IVF/ICSI and expectant management programs, are in descending order female age, underlying cause of infertility, and time trying to conceive. Expectant management programs are generally considered to be most applicable to couples with unexplained infertility, so it is this group that is used for further analysis.

The DuoFertility programme compared to IVF – Segmented by female age
Figure 2 presents the clinical pregnancy rate for both IVF/ICSI patients, and that from the use of DuoFertility for a 12-month period, each broken down by female age.

The DuoFertility programme compared to IVF – Segmented by time trying to conceive
Figure 2 presents the clinical pregnancy rate for both IVF/ICSI patients, and that from the use of DuoFertility for a 12-month period, each broken down by time trying to conceive, or time since last pregnancy, whichever is the shorter.

Statistical analysis

The R statistical package was used for all data analysis (12).
Clinical pregnancy rates for a single cycle of IVF/ICSI from the HFEA 2008 dataset were calculated assuming that any indication of pregnancy or birth was included, and the definition of unexplained infertility used was lack of any indicated cause of infertility for either partner (as opposed to patient-unexplained, i.e. no identified female factor, as present in the raw data). Clinical pregnancy rates for DuoFertility were calculated over a 12-month period of continuous use via the Kaplan-Meier estimator to account for right-censoring of data. Pregnancy was patient-reported, so although it is probable that some under-reporting occurred, it seems unlikely that a patient would falsely report as pregnant.

Results and discussion

IVF/ICSI Clinical Pregnancy Rate – Segmented by female age and cause of infertility
The HFEA 2008 dataset, segmented by both age and cause of infertility, is presented in Figure 1, which demonstrates that IVF/ICSI clinical pregnancy rates do not differ significantly between underlying causes of infertility when grouped by female age, at the 95% level. It is therefore reasonable to suppose that both cases of unexplained infertility, mild male or female factors, such as those included in the DuoFertility inclusion criteria (10), all would have substantially similar IVF pregnancy rates.

The DuoFertility programme compared to IVF – Segmented by female age
The clinical pregnancy rate from the HFEA 2008 dataset (all causes of infertility) are presented in Figure 2, alongside the clinical pregnancy rate from 12 months use of DuoFertility, segmented by female age. Clearly, greater numbers of patients are needed in the DuoFertility group to reduce the magnitude of the error bars and draw firmer conclusions; however the data suggests that DuoFertility is similarly effective as IVF for patients suffering from unexplained infertility or mild male- or female- factors, and who are aged less than 45 years at the commencement of use.

The DuoFertility programme compared to IVF – Segmented by time trying to conceive
Figure 3 presents the HFEA 2008 dataset (all causes of infertility) and DuoFertility dataset segmented by time trying to conceive/time since last pregnancy

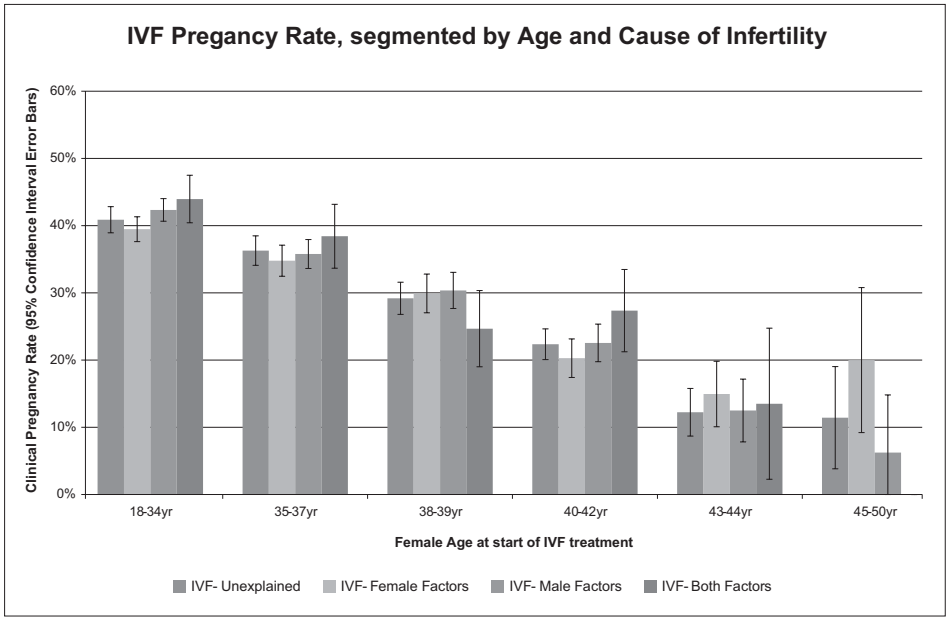

Fig. 1 - HFEA 2008 dataset, segmented by both age and cause of infertility.

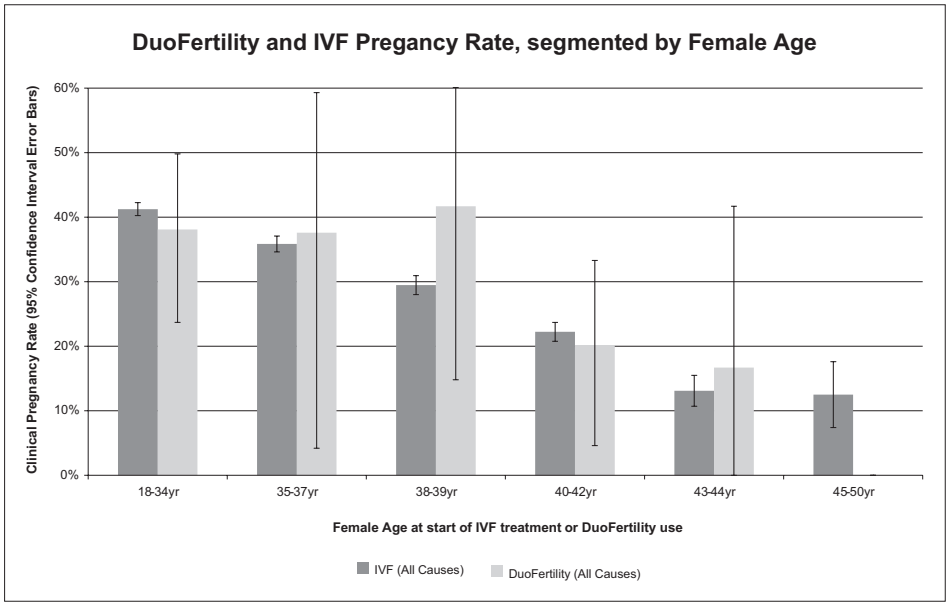

Fig. 2 - IVF/ICSI clinical pregnancy rate, segmented by female age, alongside the clinical pregnancy rate for DuoFertility.

(whichever is the shorter). This data suggests that the DuoFertility expectant management programme has similar effectiveness to IVF for patients who have been trying for less than 5 years; however again the strength of any such comparison is limited by the magnitude of the error bars.

Conclusion

Published studies comparing expectant management to assisted reproductive technologies are relatively few in number, and small in scale ([10] having 437 participants and [11] having 242 participants). However, the data presented here argues strongly for considerably more research to be undertaken in this area, given the remarkable similarity in clinical pregnancy rates observed between costly and invasive assisted reproductive technologies, and the considerably lower cost and non-invasive use of expectant management programmes.

The data presented implies that over 50% of all IVF non-donor cycles would meet the inclusion criteria for DuoFertility (11), and that the prognosis for these cou-

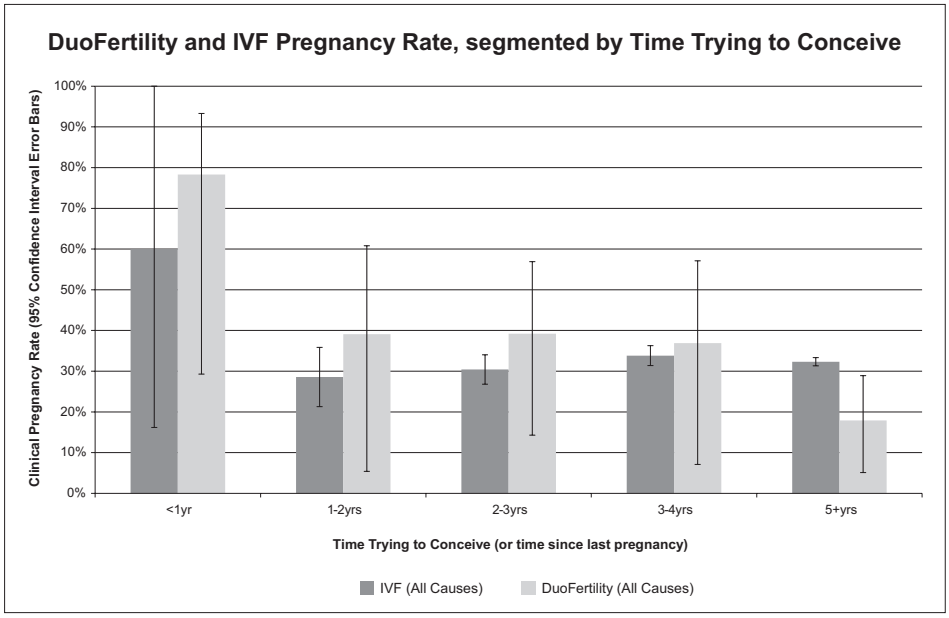

Fig. 3 - HFEA 2008 and DuoFertility data were segmented by time trying to conceive/time since last pregnancy (whichever is the shorter).

ples would be similar using DuoFertility for 12 months compared with a cycle of IVF. This also implies that, of the approximately 125,000 UK couples diagnosed as infertile each year, more than 80% would be suitable for such an expectant management approach.
It is of course vital to ensure that patients not suitable for expectant management (i.e. sterile) are provided with assisted reproduction as rapidly as possible, and it is hoped that increased use of expectant management for infertile couples will ensure that rapid provision of assisted reproductive services to those who need it most is more achievable within existing economic constraints.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Luteal phase variability in the infertile woman

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Introduction

A woman's cycle is comprised of the follicular phase (between the start of menstruation and ovulation) and the luteal phase (between ovulation and the day preceding the start of the menstruation). The maturation of the ovarian follicles and the release of the egg, controlled by pituitary gonadotrophins govern the length of the follicular phase. On the other hand, the corpus luteum is dependent on the support of the pituitary gonadotrophins during the luteal phase and the level of these hormones determines the length of this phase (1-3). In the event of a pregnancy the luteolysis is prevented by the increased human chorionic gonadotropin (hCG) level produced by the placenta (4). Several studies have looked at the general population to assess variations in menstrual cycle length and variation in the phases of the cycle (5,6). These have shown that cycle and phase variations have been associated with particular conditions such as breast cancer (7), cardiovascular diseases (8,9), and recurrent miscarriages (10-12). It has been established that some parameters correlate with the cycle and phase lengths such as age (13-17), number of children (18), lifestyle factors (19-21) and BMI (22).
It is generally agreed that the timing of ovulation is highly variable both within and between women (6,23). This causes follicular phase variability, which is well accepted (24). However the luteal phase is generally thought to be relatively consistent both within and between patients with ovulation triggering a 14-day window of high progesterone (25). This study considers the validity of this hypothesis in a group of infertile women contributing to 4799 cycles included in this study.

To date there have been no large-scale studies looking at this luteal phase variability in the infertile population (26). Using a home fertility monitor which confirms ovulation allows the assessment of a large number of women who can be followed up for several cycles.

Materials and methods

Information was collected from 923 infertile women (5168 cycles) who took part in the DuoFertility programme. All cycles for which ovulation did not occur were then removed. This left 4854 cycles from 911 women. The average number of cycles studies studied per women is 5.4 (with a range of 1 to 27 cycles).
The DuoFertility monitor (Cambridge Temperature Concepts Ltd, UK) allows the user to record menstruation, as well as other fertility information such as perceived cervical mucus secretion, urinary LH test results and ovulation pain. Women also wear a small axillary sensor that takes thousands of temperature measurements every day, as well as heat flow and movement. This information is used by the DuoFertility proprietary algorithms to predict the fertile days for couples allowing them to plan sexual intercourse during their most fertile days. The monitor also provides the women with a retrospective confirmation of the ovulation date, which is of interest in the present study. The sensitivity of the DuoFertility monitor for determination of ovulation date compared to ultrasound scanning has been shown to be >80% over a 24 hour window (27). All the information from the user is automatically transferred to the DuoFertility Fertility Centre for further analysis and advice. The couples also provide medical information such as time spent

trying to conceive, age, BMI and known fertility conditions of both partners.
Inclusion criteria for this study were patients aged 20 to 50, with a BMI of 17 to 57, and who were diagnosed with infertility (trying to conceive for more than a year with regular intercourse). All anovulatory cycles and cycles during which a pregnancy has been established (either by a home pregnancy test or hCG blood test) were excluded from the analysis.
The data was analysed using the R statistical analysis system (28). Johnson distribution (29) curves were fitted to the data using the *SuppDists* package (30). Comparisons of variance within and between the groups was performed using linear mixed effects modelling from the *nlme* package (31).

Results and discussion

The women taking part in the DuoFertility programme have a greater variability in overall cycle length, follicular phase and luteal phase lengths than is generally reported in the literature. As shown in Figure 1, the distributions of cycle length, follicular length and luteal length are similarly spread. The cycle length distribution follows a Johnson distribution with parameters γ=-0.58, δ=1.08, ξ=26.33, λ=2.84; the follicular phase follows a Johnson distribution with parameters γ=-0.94, δ=1.39, ξ=11.99, λ=3.42; and the luteal phase follows a Johnson distribution with parameters γ=0.12, δ=2.35, ξ=13.12, λ=5.94. This finding indicates that infertile women have more variable cycles than the general population, and that the variability is not restricted to just the follicular phase. This may be due to the fact that infertility is often linked with hormonal imbalance, which therefore could cause both phases of the cycle to be less regular. A standard deviation of over 5 days was initially observed for the luteal length distribution. This data was highly skewed by long phases – these were attributed to cases of unreported miscarriages. Accordingly, it was decided to right-sensor the luteal data, dropping luteal lengths greater than 45 days (less than 1% of the total number of records). This naturally caused an apparent drop in luteal length variance.
Cycle variability between women is accepted, but in the general population, the variation in cycle length for a given woman is relatively small. In our study we have shown that the variability of both phases is similar both between and within women in the infertile population. In this study, the relative contribution to variability in length of a cycle/phase was assessed (Table 1). It was observed that both inter- and intra-woman cycle variability needed to be considered when analysing the data.
In this study, patients are classified into female factor infertility (including dual-factor infertility) versus non-female factor (unexplained and male-only factor infertility). There is a shift towards longer cycles (and the follicular phase in particular) for women in the female factor group, and the distribution becomes more highly skewed; this is expected as this group contains many patients with mild PCOS (polycystic ovarian syndrome), who have irregular cycles. The added effect of splitting the data by female factor on cycle/follicular/luteal variability was also investigated (Table 2). It was observed that, while this had some contribution to cycle and follicular variance, nearly all the contribution of luteal variance was again due to intra-woman variability.

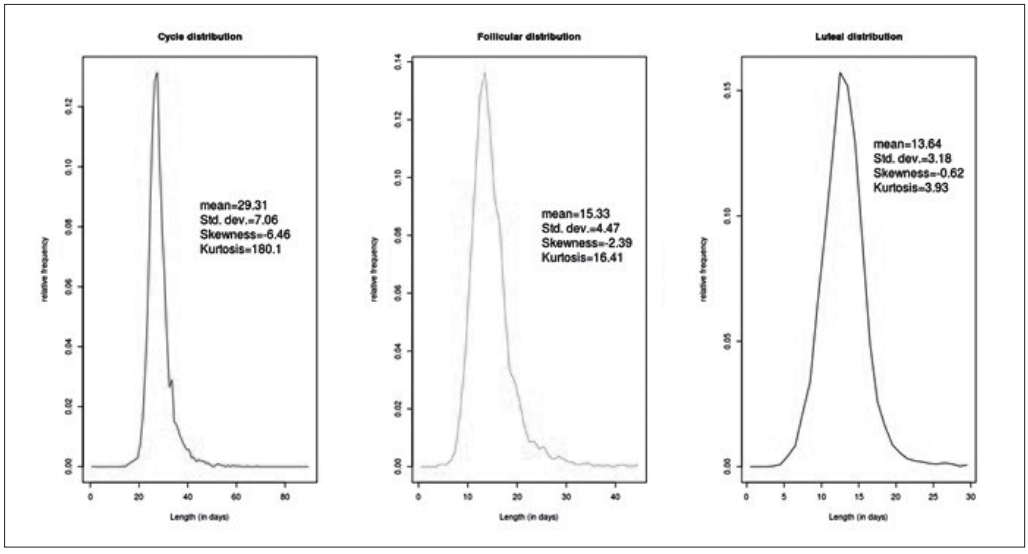

Fig. 1 - Overall distribution of observed cycle, follicular/luteal lengths.

TABLE 1 - RELATIVE CONTRIBUTION OF VARIABILITY OF A CYCLE/PHASE.

	Mean (days)	Standard Deviation (days)	% variability due to inter-woman effects	% variability due to intra-woman effects
Cycle	29.31	7.06	49	51
Follicular	15.33	4.47	48	52
Luteal	13.64	3.18	31	69

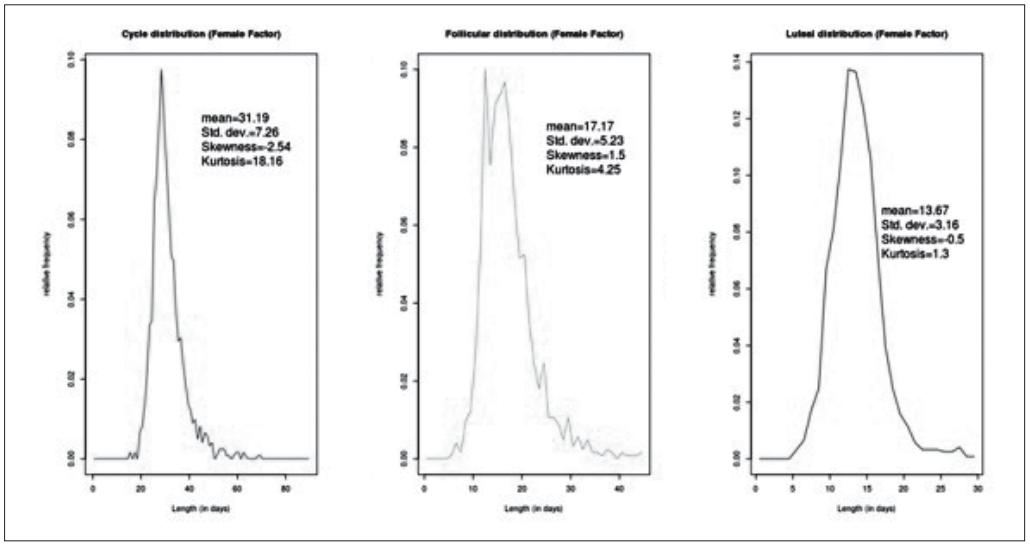

Fig. 2 - Distributions of cycle/follicular/luteal lengths, female factor.

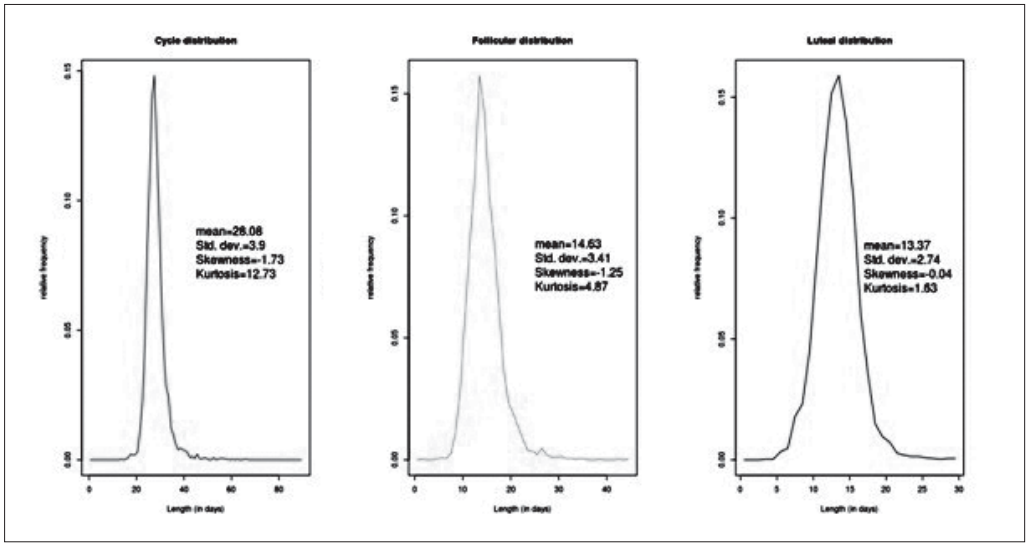

Fig. 3 - Distributions of cycle/follicular/luteal lengths, non female factor.

TABLE 2 - RELATIVE CONTRIBUTION OF VARIABILITY OF A FACTOR/CYCLE/PHASE.

	Mean (days)	Variance (days)	% variability due to factor effects	% variability due to inter-woman effects	% variability due to intra-woman effects
Cycle	29.31	7.06	17	37	46
Follicular	15.33	4.47	15	37	48
Luteal	13.64	3.18	1	18	81

Particular attention should be drawn to cases of longer luteal phase, as these may result from implantation which doesn't lead to a successful ongoing pregnancy. The cycles during which a positive pregnancy test has been recorded were excluded from the analysis; however some women who have been trying for several years do not want to take a pregnancy test, as this increases their stress level. It is therefore likely that some of these cycles represent pregnancy followed by early miscarriage.

As part of the infertility workup in primary care settings, women frequently undergo a blood test on the 7th day after ovulation. The test typically requires a progesterone level of at least 30 nmol/L to record confirm that ovulation has occurred, which is generally the case between 3.5 and 9 days post ovulation (5,6). This test is generally performed on day 21 of the menstrual cycle for convenience; however our findings suggest that this test will be taken at a time leading to erroneous results in 42% of cases (in 25% of cases prior to ovulation or fewer than 3 days after ovulation, and in 17% greater than 9 days after ovulation).

Conclusion

This is the first large-scale monitoring study for cycles with infertile women in the home. This has allowed us to identify a difference in cycle length distribution and phase length distribution in infertile women, and contrast this with the results of earlier studies on the general population.

These findings highlight that the cycles of infertile women are frequently different to those of the general population. Given that the variation of the cycles for women who have unexplained infertility is also greater than that reported for the general population, this may indicate that a significant proportion of these women have an underlying hormonal imbalance which may require further investigation.

These findings also highlight the potential problem linked with using "7 days before the usual end of the cycle" or, worse, "day 21 after the start of the cycle" as the standard day of testing for progesterone level rather than identifying ovulation and actually testing 7 days post-ovulation, as for many infertile women this will lead to a potentially erroneous result (up to 42% of the cycles in our study).

If facilities to monitor date of ovulation are not available, an alternative strategy is simply to perform the progesterone test at some pre-determined point in the cycle (for example, day 21), and then to discard the result if the onset of menses is not within 5 to 11 days of the date of progesterone measurement. Our data suggests that this will be the case in a significant proportion of infertile women, and furthermore, a very high fraction of those for whom the sampling date was inappropriate will continue to be problematic without implementing some form of ovulation monitoring. It should also be noted that this strategy ignores the considerable variability in luteal-phase length, which has been shown to be almost entirely intra-woman in nature.

Therefore, wherever possible, ovulation monitoring should be utilised to confirm the date of ovulation prior to progesterone measurement.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Anti-Mullerian hormone in patients with polycystic ovary syndrome

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Introduction

The polycystic ovary syndrome (PCOS) is an important cause of both menstrual irregularity and androgen excess in women. It is a common endocrinopathy, occurring in 5 to 7 percent of reproductive age women. When fully expressed, the manifestations include irregular menstrual cycles, hirsutism / biochemical hyperandrogenism (HA), and polycystic ovary morphology (PCO). In the adult ovary, antimullerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles and negatively regulates folliculogenesis. AMH is overproduced in the polycystic ovary and was recently proposed to play a role in the ovulatory dysfunction of polycystic ovary syndrome (an increased AMH tone within the cohort could be involved in the follicular arrest of PCOS).

Objective

To explore the serum AMH level in young adult patients with polycystic ovary syndrome (PCOS), and to evaluate its diagnostic value for PCOS.

Patients and methods

Serum AMH was measured in a cohort of 120 patients with PCOS and 79 age-matched controls (80 young adult patients with PCOS and 60 age-matched controls 18-35 yrs). Diagnosis of PCOS was established on the basis of Rotterdam criteria after an exception of other diseases. AMH levels were measured in the samples using immunoenzymatic assay. Its diagnostic potential was evaluated by Receiver Operating Characteristic (ROC) curves.

Results

The serum level of anti-Mullerian hormone (AMH), a product from granulosa cells involved in follicle growth, has been shown to correlate tightly with the volume and small antral follicle number (FN) at ultrasonography (US) in controls women. Because PCOS is associated with increase in growing FN, we investigated whether an increased AMH serum level in PCOS patients. The serum AMH level was higher in young adult PCOS patients than in controls (6.2±3.4 ng/mL vs. 3.9±2.2 ng/mL, $p=0.0003$), and positively related to the mean ovarian volume in PCOS patients ($r = 0.3$, $p<0.05$). The best compromise between specificity (76%) and sensitivity (63%) was obtained with a cut-off value of 8 ng/mL. No difference was found between AMH levels in the cohort 36-60 yrs in both groups.

Conclusions

Serum AMH levels are elevated in young adult patients of PCOS. Serum AMH measurement offers a relatively poor diagnostic potency with a sensitivity of 63% and a specificity of 76% at 8 ng/mL.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Abdominal adiposity correlated with systemic inflammatory status. A risk for endometrial cancer

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Introduction

Obesity is an endemic disease of the 21st century, with a continuously increasing prevalence, particularly in young persons. In economically developed countries, endometrial cancer is associated with obesity in a proportion of 40% (1). In the context of endometrial cancer, visceral obesity as a risk factor is associated with a chronic inflammatory process, confirmed by the increase of inflammatory markers, CRP, IL-6, TNF-α, in the systemic circulation of obese patients (2). This chronic proinflammatory state is in its turn a risk factor for endometrial cancer.
Adipose tissue is no longer considered just an energy storage organ, but a real endocrine organ. The adipocyte is the central element that integrates multiple metabolic and endocrine signals.(3) This cell represents the source for a multitude of bioactive peptides, markers of the systemic inflammatory syndrome, IL-6, TNF-α, leptin, adiponectin (4).
Systemic IL-6 and TNF-α levels increase with age and body mass index. The action of TNF-α is mediated by a complex of cytokines and hormones including IL-6 and leptin (5). Obesity is associated with an increased IL-6, leptin, as well as TNF-α expression (6).
The use of dual X-ray absorptiometry (DXA) for the determination of body composition as a whole as well as by regions has started to be increasingly used. The radiation exposure resulting from the scanning of the entire body is relatively minor (~0.3 μSv) and represents less than the unavoidable daily background radiation exposure (~2.0 μSv/ 24 hours).
The study aims to evaluate the presence of a correlation between abdominal adiposity assessed by DXA and systemic inflammatory status expressed by IL-6 and TNF-α in patients diagnosed with endometrial cancer.

Materials and methods

The study is a case-control analysis that includes 2 groups of patients: *group I* – 44 patients diagnosed with endometrial cancer, *group II* – 44 patients without gynecological pathology or inflammatory disorders (control group).
The diagnosis of endometrial cancer was made after histopathological examination that analyzed the tissue material obtained following endometrial biopsy. Endometrial biopsy was performed in the case of considerable metrorrhagia, in the case of metrorrhagia during climax, as well as in the case of aspects undetected by US (endometrial thickness and vascularization).
After the performance of clinical examination and anthropometric measurements (BMI, abdominal circumference - AC), the patients underwent DXA (GE, Prodigy Lunar), which measured the amount of abdominal adipose tissue. BMI was calculated by the formula BMI = weight (kg)/ [height (m)]^2. The height of the subjects was accurately measured, using an anthropometer fixed to the wall (222 model; Seca GMBH, Germany). Body mass was determined with an accepted error of 20 g, using a calibrated electronic scale (FW-150K model; A&D Mercury). AC (cm) was measured in orthostatism, at umbilical level.
Abdominal adipose tissue was obtained by total body scans. The "abdominal region" was defined using the software provided by the manufacturer, with the upper limit at the junction of the lower 1/5 with the upper

4/5 of the pelvis-to-chin distance, and the lower limit at the upper part of the greater trochanters.
From each subject included in the study, 6 ml fasting blood were taken by venous puncture and collected in test tubes without anticoagulant in order to determine plasma IL-6 and TNF-α levels. The serum obtained by centrifugation was divided and stored in 600 µl freezing tubes at a temperature of -30^0 C until tests were performed, in order to avoid repeated freezing-thawing cycles.
The serum IL-6 concentration was measured by the sandwich ELISA technique using the Human IL-6 Immunoassay HS600B kit, R&D Systems USA. The serum TNF-α level was measured by the sandwich ELISA technique using the Human TNF-α Immunoassay HSTA00D kit, R&D Systems USA.
The informed consent of all patients was obtained.
All parameters were included in the study database and version 13 of the SPSS software and *Microsoft Excel with Analysis Tool Pack* were used for statistical analysis. The Kolmogorov Smirnov test was applied for the testing of normal distribution. The Student t test was used for the comparison of the means and the Mann-Whitney test for rank comparison in two independent samples.
The study compares the two groups regarding abdominal adiposity assessed by DXA and aims to evaluate the presence of a correlation between adiposity located at abdominal level and systemic inflammatory status expressed by plasma IL-6 and TNF-α levels.

Results

The patients of the control group had on DXA examination a mean abdominal fat amount of 10680.27±3675.49 g (with limits between 17156.00 and 63194.00 g), while in the group of patients with endometrial cancer, the mean abdominal fat amount was 21616.28±4758.59 g (with limits between 1887.00 and 21110.00 g). Thus, there was a statistically significant difference in abdominal fat (p<0.0001) between the two groups (Fig. 1).
The ROC curve for the identification of the cut-off value for abdominal fat assessed by DXA is shown in Fig. 2. The area under the curve was 0.97, *p<0.00001*. The cut-off value identified was 16324 g. A DXA abdominal fat value higher than 16324 seems to be a cut-off value for patients with endometrial cancer (*p<0.00001*).
Inflammatory status expressed by IL-6 and TNF-α was significantly higher in the group with endometrial cancer compared to the control group. Thus, the plasma IL-6 level in the group with endometrial cancer was 26.98±23.56 pg/ml (with limits between 5.95 and 83.48 pg/ml) compared to the control group, 11.22±6.79 pg/ml (with limits between 2.80 and 26.92 pg/ml). The plasma TNF-α level was 4.18±2.23 pg/ml (with limits between 1.83 and 10.25 pg/ml) compared to the control group, 1.54±0.48 pg/ml (with limits between 0.60 and 2.93 pg/ml) (Fig. 3, 4). Abdominal fat is in a positive linear correlation with the plasma IL-6 level (Fig. 5). The correlation coefficient between abdominal fat and IL-6 was **R=0.43 p=0.0001**, indicating a significant correlation. 15% ($d=r^2=0.15$) of the IL-6 value is due to abdominal fat. Abdominal fat is in a positive linear correlation with the plasma TNF-α level (Fig. 6). The correlation coefficient between abdominal fat and TNF-α was **R=0.73 p=0.000001.** This coefficient indicates a significant correlation between abdominal fat and the

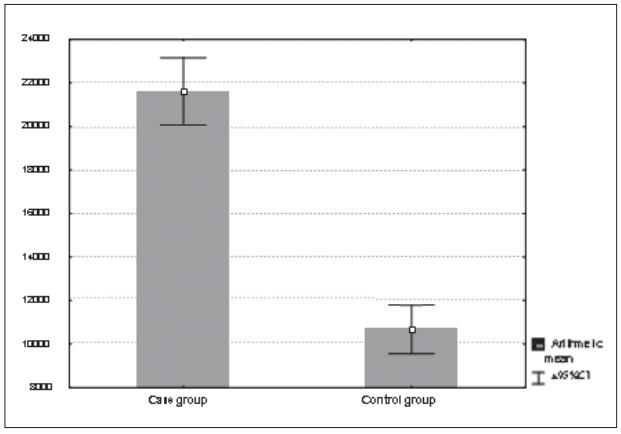

Fig. 1 - Comparison of abdominal fat assessed by DXA between the two groups.

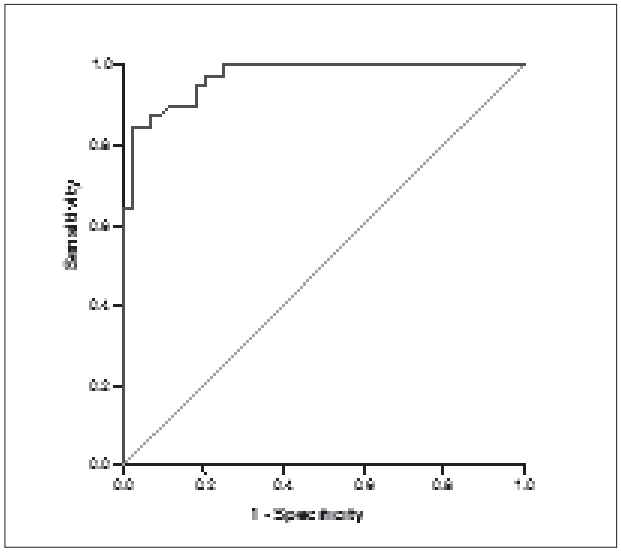

Fig. 2 - ROC curve for the identification of the cut-off value for abdominal fat.

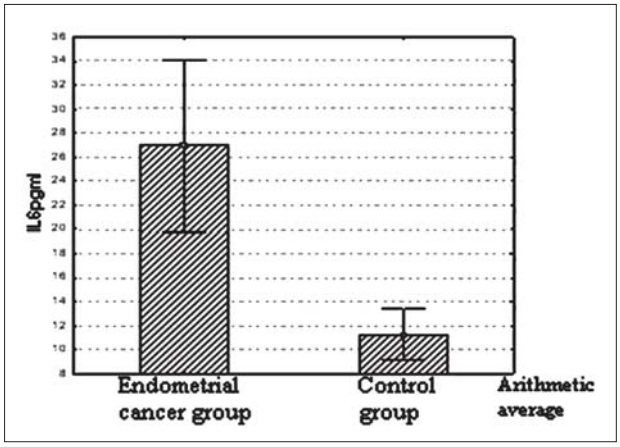

Fig. 3 - Comparison of IL-6 between the two groups.

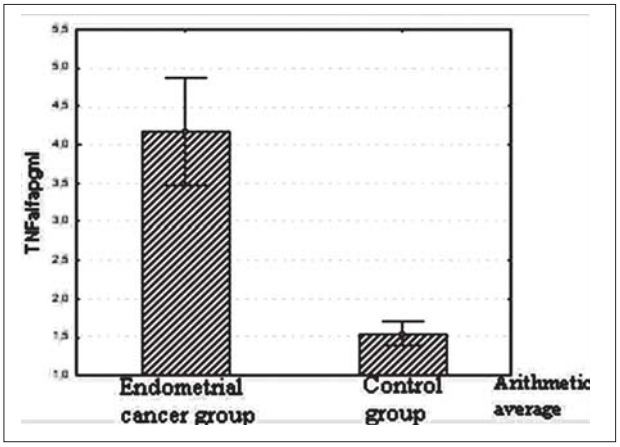

Fig. 4 - Comparison of TNF-α between the two groups.

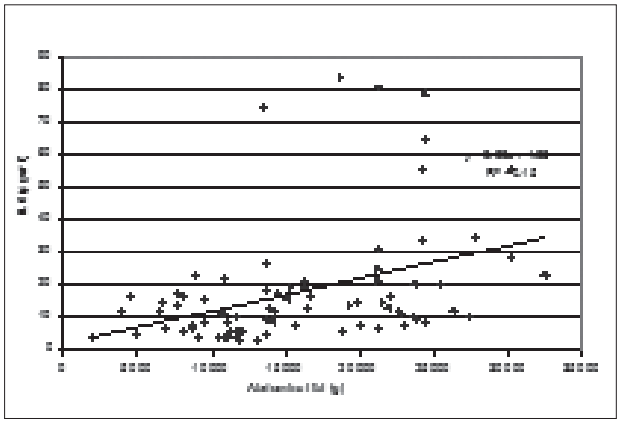

Fig. 5 - Correlation between IL-6 and abdominal fat.

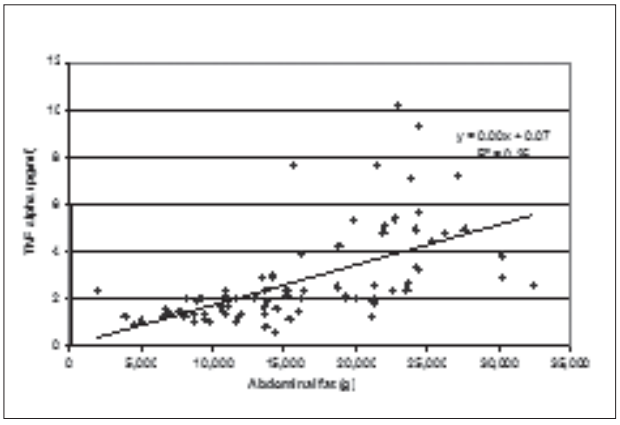

Fig. 6 - Correlation between TNF-α and abdominal fat.

plasma TNF-α level. 35% ($d=r^2=0.35$) of the TNF-α value is due to abdominal fat.

Following multivariate analysis, it can be stated that **abdominal fat** assessed by DXA is the dominant parameter, which influences plasma IL-6 and TNF-α levels.

Discussion

Adipose tissue, accumulating in various anatomical areas (regional adiposity) may have unique characteristics related to the different expression of the enzymes and receptors involved in triglyceride synthesis, lipolysis and adipokine synthesis. Consequently, it is essential to study the relationship between regional obesity and systemic inflammatory status and to identify the role of systemic inflammatory status in the mediation of the metabolic effects of regional adiposity. So far, regional obesity and its correlation with systemic inflammatory status have not been well documented. This study supports the idea that abdominal adipose tissue is correlated with systemic inflammatory status expressed by IL-6 and TNF-α.

Anthropometric indices evidenced strong correlations with systemic inflammatory status. However, the value of these indices for the explanation of systemic inflammatory status was diminished after the adjustment for DXA indices. Thus, although BMI and AC can be systemic inflammation markers, DXA indices have a better prediction in assessing systemic inflammatory status (7).

In this study, abdominal adiposity assessed by DXA examination is strongly correlated with inflammatory status expressed by plasma IL-6 and TNF-α levels. The accumulation of lipids is favored in the lower part of the body in premenopausal women compared to men (8). This can be due to lipoprotein lipase activity, a key factor responsible for the release of adipocyte lipolytic products for their storage as TG (9). On the other hand, the adipose cells of the lower part of the body

have a lower lipolytic response to catecholamines and a higher response to insulin-mediated lipogenesis than visceral adipocytes. Also, adipose cells in the lower body have a lower beta 1 and beta 2 adrenoreceptor density, as well as a higher alpha 2 adrenoreceptor affinity (10). As a result, the adipose cells of the lower part of the body in women are lipolysis-resistant and predisposed to lipogenesis. This can explain the predominance of gynoid fat distribution in the female sex, which is correlated with the reduction of coronary as well as oncological disorders (endometrial cancer, breast cancer, colorectal cancer). In this study, abdominal fat is positively correlated with endometrial cancer. Obesity increases systemic exposure to free estrogens via the aromatization of androgens in visceral adipose tissue, via the reduction of SHBG production, via the reduction of serum progesterone by anovulation, via the proinflammatory state (11). The relationship between endogenous steroid hormones and the risk for endometrial cancer is described as the *"unopposed estrogen"* theory (12). According to this theory, the risk of endometrial cancer increases in patients with high serum estrogen and/or low progesterone levels. This study supports the idea that in patients with endometrial cancer abdominal fat content is significantly higher compared to the control group. A DXA abdominal fat value higher than 16324 g seems to be a cut-off value for patients with endometrial cancer.

Conclusion

Considering that abdominal fat is positively correlated with the incidence of endometrial cancer and that it is, at the same time, the dominant parameter, which influences plasma IL-6 and TNF-a levels, it can be interpreted that abdominal fat is a risk factor for endometrial cancer through systemic inflammatory status.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

D-Chiro-Inositol treatment in patients with polycystic ovary syndrome

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Introduction

Polycystic Ovary Syndrome (PCOS) is a complexdisease characterized by various endocrinedisorders that can be the potential cause ofanovulation and hyperandrogenism condition.
This heterogenoussyndrome affects about 5-10% of reproductive age female population, and it can be considered as the most common endocrine disorder affecting women during reproductive life (1).
PCOS is a multifactorial polygenic disease: are interested genes involved in insulin-resistance, genes that encode inflammatory factors, genes that regulate steroidogenesis and folliculogenesis.
Chronic anovulation most often manifests as oligomenorrhea; anovulatory cycles may lead to dysfunctional uterine bleeding and decreased fertility. Cutaneous manifestations of hyperandrogenemia in the polycystic ovary syndrome include hirsutism, acne, and male-pattern hair loss (androgenic alopecia), whereas acanthosisnigricans is a cutaneous marker of hyperinsulinemia.
A substantial proportion of women with the polycystic ovary syndrome are overweight; many are obese, others don't reach high level of obesity. Although obesity itself is not considered the inciting event in the development of the syndrome, excess adiposity can exacerbate associated reproductive and metabolic derangements (2).
D-chiro-inositol is an important second messenger in signal transduction of insulin, intervening in the activation of serotonin receptors in the Central Nervous System.
Dietary sources of D-chiro-inositol are rather scarce. Deficiencies of D-chiro-inositol are related to some metabolic and endocrinological disorders, such as insulin resistance and polycystic ovary syndrome (PCOS).
Clinical studies have shown that low levels of D-chiro-inositol reduce insulin-sensitivity and causeHypertriglyceridemia.There are also several scientific evidences that link D-Chiro-Inositol to PCOS.The supplementation of D-chiro-inositol in women with PCOS has led to a rebalancing of insulin levels, triglycerides and cholesterol with a decreased testosterone serum levels, and reactivation of ovulation (3).
In the study conducted by Nestler were enrolled 44 women with PCOS, which were administered 1200 mg of D-Chiro-Inositol a day.
Results were: spontaneous ovulation in 86% of women undergoing treatment with D-chiro-inositol, compared to an ovulatory rate of 26% in the placebo group ($P<0.001$); significant reduction in systolic blood pressure ($P=0.05$) and diastolic blood pressure; significant reduction ($P=0.002$) in serum levels of triglycerides, Total testosterone values ($P=0.003$) and DHEA-S ($P<0.001$) in Group D-chiro vs. placebo (3).
Another study havepointedoutthat in women with PCOS, an increase of the release of DCI-IPG (D-chiro-inositol containing inositol-phosphoglycan mediators) stimulated by glucose, is correlated significantly with improved sensitivity to insulin effect.This significant correlation between DCI-IPG and insulin sensitivity suggests the hypothesis that the DCI-IPG may represent a novel therapeutic intervention in patients with PCOS (4).
The aim of our study is to evaluate the clinical, endocrine and metabolicresponse of young women with PCOS, treated for12 weeks with a therapeutic protocol D-chiro-inositol only based.

Materials and methods

All the patients were enrolled in the Institute of Obstetric and Gynecological pathology ("Santo Bambino" Hospital, Catania), at the GynecologicalEndocrinology Clinics.
In the 12-month enrollment phase a total of 58 womenwith polycystic ovary syndrome (PCOS) were selected. PCOS diagnosis was indicated by oligo-amenorrhea (six or fewer menstrual cycles during a period of one year), hyperandrogenism (hirsutism, acne or alopecia) or hyperandrogenemia (elevated levels of total or free testosterone), andtypical feature of ovaries at ultrasound scan.
The average age was 27 years with a standard deviation of 7 years; the mean BMI value was 28, W/H ratio was 0.87 m, and the mean blood pressure value was 130/86 mmHg. Acne was evaluated with the Cremoncini score and the mean value was 3.5, while the hirsutism with the Ferriman and Gallway score with the mean value of 15.
The study was double-blind designed and, according to a randomization table, patients were divided into two groups: 38 patients of Group Aintook 250 mg of D-chiro-inositol (Chirositol™, Cyvex Nutrition)in combination with manganese, folic acid and B12 vitamin 2 times aday, continuously for 3 months; manganese amplifies the response to D-Chiro-inositol (synergistic action) in relation to the improvement of the insulin-sensitivity and B12 vitamin is used as therapeutic coverage in the event of combination with metformin (which tends to induce B12 deficiency in long-term protocols).
Instead, the 20 patients of Group Bintookan aspecific multivitamin as placebo twice a day for 3 months. No significant differences were found between the two groups in mean age and body mass index (BMI).
Three months after the treatment was carried out a clinical, endocrine and metabolic assessment of patients enrolled.
Clinical evaluation: BMI, W/H Ratio, acne score, hirsutism score, blood pressure value, relief of the number of spontaneous menstrual cycles and side effects.
Endocrine evaluation: Serum assay (2 samples) of LH, FSH, E2, total and free testosterone, androstenedione, DHEA-S, 17-OHP, SHBG, prolactin, TSH, fT3, fT4 (withdrawals made within the 8 th day of spontaneous or progesterone-induced cycle).
Metabolic evaluation: glycemia, total and fractionated cholesterol, serum triglycerides, BUN and Basal insulin.

Results

The comparison between Group A and Group B was performed using:
– Student *t* test for quantitative data normally distributed (age, BMI, W/H ratio, blood pressure, hormones, cholesterol and triglycerides serum levels);
– U test for quantitative data not normally distributed (number of patients with regular menstrual, hirsutism and acne score.

From a clinical point of view, our study has highlighted a significant retrieval of menstrual-cycle regularity ($p<0.001$) (Fig. 1) in a rate higher than 60% in patients treated and a significant improvement of acne score($p<0.05$) (Fig. 2) in patients of Group A in treat-

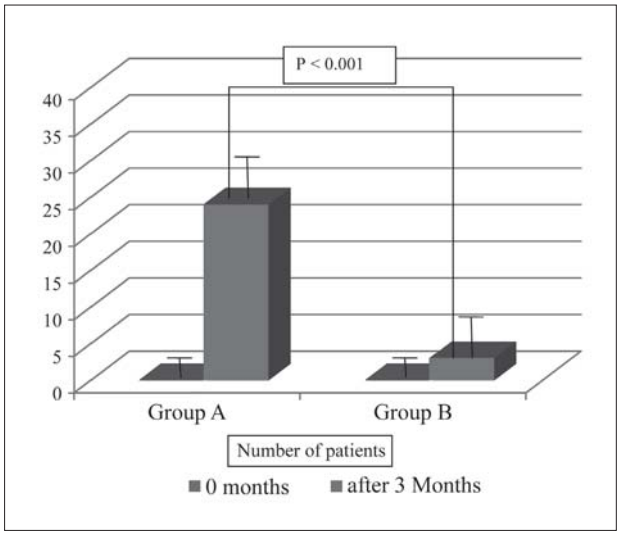

Fig. 1 - Retrieval of menstrual-cycle regularity before and after treatment with D-Chiro-Inositol (Group A) and placebo multivitamin (Group B).

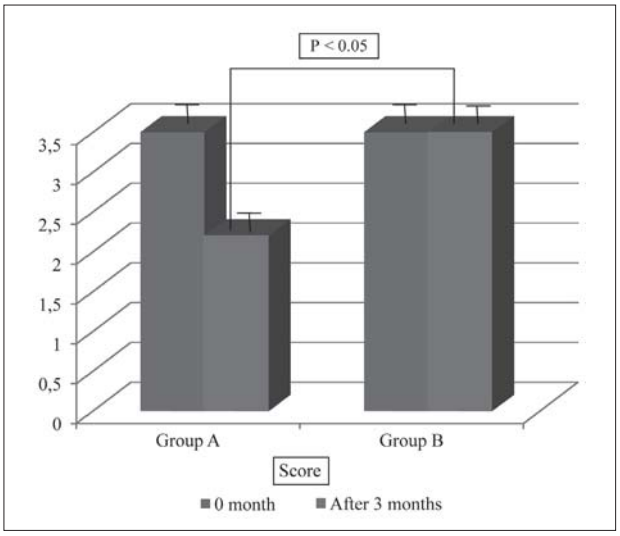

Fig. 2 - Acne Score before and after treatment with D-Chiro-Inositol (Group A) and placebo multivitamin (Group B).

ment with D-chiro-inositol. No significant adverse effects were reported.
With regard to endocrine evaluation, there was a significant decrease of DHEA-S serum levels in the Group treated with D-chiro-inositol ($p<0.05$). A trend of decreasing of all other hormones tested was noticed, but there was no significant changes.
Metabolic evaluation found a significant decrease of triglycerides ($p<0.05$) and basal insulin serum levels ($p<0.05$) (Fig. 3) in patients treated with D-chiro-inositol.
A trend of decreasing of all metabolic parameters evaluated was noticed, but there was no significant changes.

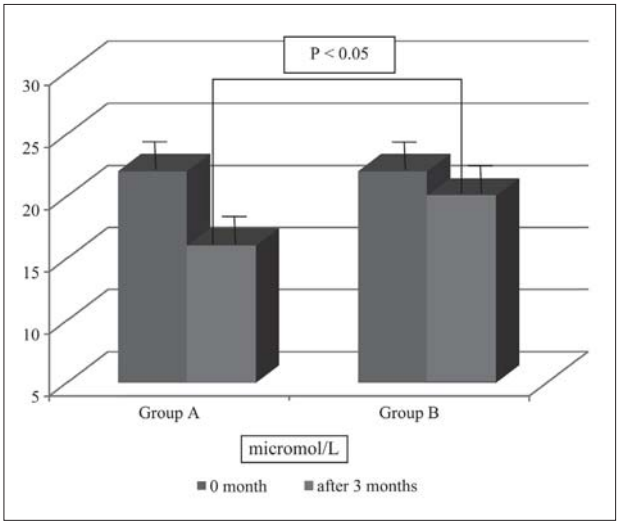

Fig. 3 - Basal insulin serum level before and after treatment with D-Chiro-Inositol (Group A) and placebo multivitamin (Group B).

Discussion

There are several scientific evidences that show the connection between D-chiro-inositol and PCOS.
Some actions of insulin are mediated by putative inositolphosphoglycan mediators, and a deficiency in D-chiro-inositol-containing inositolphosphoglycan (DCI-IPG) may contribute to insulin resistance in women with polycystic ovary syndrome (PCOS) (5).
It was shown that PCOS patients have increased urinary clearance of DCI (uClDCI), which was associated with hyperinsulinemia (6,7).
D-chiro-inositol is an important second messenger in signal transduction of insulin, intervening in the activation of serotonin receptors in the Central Nervous System.
Dietary sources of D-chiro-inositol are rather scarce.
The supplementation of D-chiro-inositol in women with PCOS has led to rebalancing of insulin levels, triglycerides and cholesterol with a decreased testosterone serum levels, and reactivation of ovulation (3).
In a recent study it was explained, therefore, that the D-chiro-inositol-to-myo-inositol ratio is regulated by an insulin-dependent epimerase. Enzyme activity varies among tissues, likely owing to the specific needs of the two different molecules. An Italian group of study hypothesizes that in the ovaries of polycystic ovary syndrome patients, epimerase activity is enhanced, leading to a local myo-inositol deficiency which in turn is responsible for the poor oocyte quality (8).
This clinical trials carried out highlights the role of D-chiro-inositol in the ovarian function of patients with polycystic ovary syndrome, in particular we must emphasize its importance in clinical restore of normal menstrual cycle, which is often only the tip of the iceberg of the polycystic ovary syndrome (9), and improvement of acne.
From a clinical point of view, our study has highlighted a significant retrieval of menstrual-cycle regularity in a rate higher than 60% in patients treated and a significant improvement of acne score in patients in treatment with D-Chiro-Inositol.
With regard to endocrine evaluation, there was a significant decrease of DHEA-S serum levels in the Group treated with D-Chiro-Inositol; a trend of decreasing of all other hormones tested was noticed, but there was no significant changes.
Moreover, metabolic evaluation found a significant decrease of triglycerides and basal insulin serum levels in patients treated with D-Chiro-Inositol. No significant adverse effects were reported.
Our study proceeds with the recruitment of other patients and monitoring of women previously enrolled also at 6 and 12 months after the beginning of therapy.
Furthermore, preliminary data suggest an improvement of the therapeutic action of D-Chiro-Inositol if administered in higher doses: we recommend an average dose of 1000 mg daily.
For these reasons, the D-chiro-inositol could be added to valid PCOS treatment protocols, as further explained below:
– Treatment with only D-chiro-inositol in regular weight, with oligomenorrhea (or secondary amenorrhea) and low grade acne. In lean women with the polycystic ovary syndrome, D-chiro-inositol reduces circulating insulin, decreases serum androgens, and ameliorates some of the metabolic abnormalities (increased blood pressure and hypertriglyceridemia) of syndrome X (10).
– Treatment with low-calories diet, D-chiro-inositol

+ metformin in patients with insulin resistance, non- or poorly symptomatic and oligomenorrhea or secondary amenorrhea (5).
– Treatment with low-calories diet, D-chiro-inositol + Metformin + Hormone Therapy and / or Anti-androgens in symptomatic patients, obese or overweight, with insulin resistance (5).

Declaration of interest
The authors report no conflicts of interest.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The role of androgen supplementation in the improvement of IVF success rates in older women - A 5 year experience

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Background

It has been twelve years since the first report suggesting that exogenous androgens could improve ovarian response in those women classified as having diminished ovarian reserve (DOR) (1).Despite the argument as to the definition of ovarian reserve (OR) (2), it is generally accepted that women over the age of 40 will have declining ovarian reserve and that this is the major limiting factor to their success with assisted reproductive technology. It is important to understand DOR as women over 40 represent the most rapidly growing group of patients seeking fertility treatment (3).
It has been suggested that ovarian reserve is not static but rather dynamic from cycle to cycle, and that the diagnostic indicators of OR, basal follicle stimulating hormone (FSH), anti-Mullerian hormone (AMH) and antral follicle count (AFC) are age-specific and change in parallel with OR (4).FSH and AMH seem to be less reliable prognosticators of success. In our hands AFC and a trial of stimulation with maximum doses of gonadotrophins have proved to be the best markers of ovarian response.
Interestingly, a survey of 196 IVF units worldwide has shown that 26% of IVF units utilize dehydroepiandrosterone (DHEA) for patients with DOR (5). Since 2005 reports from Gleicher and Barad [6-8] have shown that DHEA supplementation improves ovarian response in terms of both quantity and quality of oocytes and embryos, leading to increased pregnancy rates. In addition, Gleicher and our group have shown decreased miscarriage rates from over 50% in women over age 40 undergoing IVF to 25% and less with DHEA pre-treatment. [9]. There is now direct evidence from preimplantation genetic screening that DHEA reduces embryo aneuploidy to explain these results (10,11).
At the 14th World Congress of Gynecological Endocrinology in 2010 our group presented data to show that pre-treatment of older women with DHEA followed by controlled ovarian hyperstimulation and intrauterine insemination (COH-IUI) resulted in an excellent clinical pregnancy rate of 23% per cycle. The current study was undertaken to evaluate the efficacy of DHEA pre-treatment in the same age group of older women who had failed to conceive with COH-IUI and proceeded to in vitro fertilization (IVF).

Patients and methods

A retrospective cohort study was conducted in a private university affiliated fertility centre. Despite a large intake of appropriate patients, we have been unable to conduct prospective randomized trials (RCT) with DHEA as women in this age range refuse to be randomized. From a total of over 600 patients who began treatment with DHEA from Feb. 2006 to Nov. 2011 we have ongoing data on over 400 patients, a significant number having been lost to follow up.
84 IVF cycles were performed in women between ages 40 and 46 who had been pretreated with at least 3 months of oral micronized DHEA 25 mg three times daily. We included all patients up to age 46 for analysis and then repeated the analysis after excluding patients who were age 43 and older at the time of commencing the IVF cycle. This was done in order to compare our results with those of the only RCT in the literature that also excluded patients over age 43 (12). Our inclusion criteria were based upon patients with

late maternal age who demonstrated poor ovarian follicular response to an oral aromatase inhibitor, or to COH-IUI with high-dose gonadotrophins, elevated cycle day 3 FSH levels, very low AMH levels, low antral follicle counts, and previous failed or poor stimulation for IVF at other institutions.

Informed consent for the use of DHEA was obtained through physician discussion and patient review of all published data on DHEA with respect to fertility treatment.

Side effects such as acne, hirsutism, hair loss, weight gain and sleep difficulties were rare and seldom necessitated discontinuation of DHEA (less than 2% of patients).

All patients had an endometrial biopsy in the mid-luteal phase of the immediately pre-IVF menstrual cycle in order to increase the implantation rate (13). Stimulation protocols were either a microdose GnRH agonist flare with FSH and FSH/hMG combined as described by Schoolcraft (14), or maximal dose (450 IU) rec-FSH and the addition of a GnRH antagonist. Intracytoplasmic sperm injection (ICSI) was used in all cases.

We had a significant male factor component in 33% of cases. Embryo transfer was performed by one of two operators at 72 hr. post-oocyte retrieval utilizing a double-lumen, bulb-tipped catheter assisted by transabdominal ultrasound guidance.

Results

We had 16 IVF cycles cancelled due to failure to stimulate with maximal doses of gonadotrophins and 52 cycles were completed to embryo transfer. Table I summarizes the results of the 52 cycles with respect to measured parameters in the Non-pregnant group and the Pregnant group. Unfortunately AMH (data not shown) was only obtained in 10 patients due to the recent availability and high cost of the test in Canada.

Figure 1 shows the analysis of the 52 completed IVF cycles in which there were 18 pregnancies (35%) and 34 cycles with no pregnancy (65%).

TABLE 1

	Negative (N=34) Mean ± SD	Positive (N=18) Mean ± SD	p value
Age	40.8±0.7	41.0±0.8	NS
Initial D3 FSH Level	9.5±5.1	9.8±5.5	NS
BMI	23.4±3.6	22.6±3.4	NS
Months on DHEA	9.6±8.8	10.7±7.3	NS
# Cycles on COH +/- IUI + DHEA	0.8±1.3	1.3±1.1	NS
# Eggs retrieved	6.7±4.9	10.3±6.6	0.05340
# of embryos	3.8±2.8	7.4±6.5	0.03420
# D3 embryos	3.5±2.5	6.2±5.7	NS
D3 FSH	9.5±5.0	8.9±4.6	NS
D3 E2	219.1±178.3	141.3±45.3	0.02108
Antral follicle count	4.1±3.8	7.4±5.3	0.02975
E2 at HCG	5247.6±2082.9	6257.6±1631	NS
PGN at HCG	9.0±5.4	7.8±2.5	NS
Endometrial thickness at HCG	1.0±0.2	1.1±0.2	0.05794
# of follicles at HCG	4.6±2.0	7.8±3.6	0.00197
Total hMG meds	4210.8±966.3	4113.9±959	NS

*NS= Non statistical significance.
** Statistical significant adata is shown in bold.

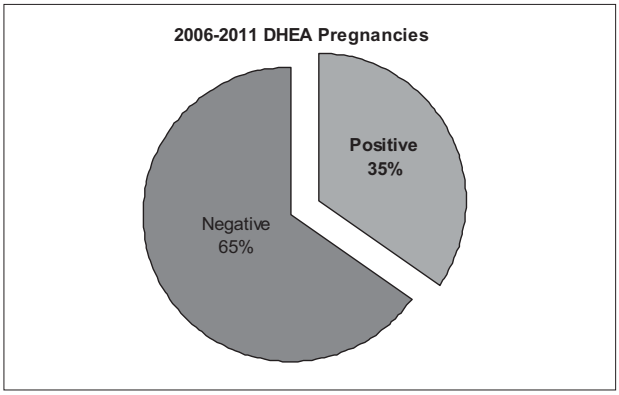

Fig. 1

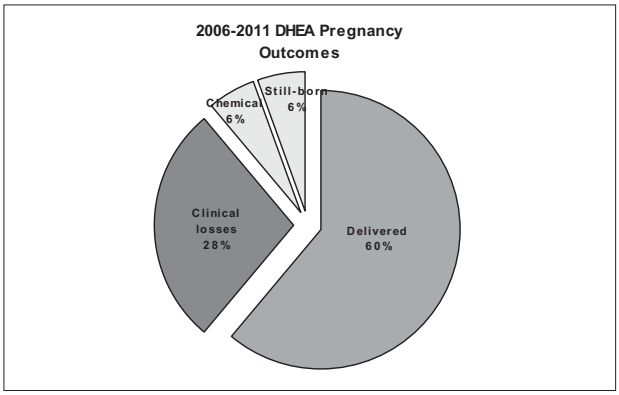

Fig. 2

Figure 2 shows the outcome of the pregnancies. There were 6 early pregnancy losses, including one chemical (33%), and 12 patients delivered as of November 2011 (67%). The clinical pregnancy rate per embryo transfer was 33% and the live birth rate per embryo transfer was 21%. One baby was stillborn and excluded from the analysis. There were 4 multiple pregnancies including one twin loss.

Conclusion

U.S. national IVF success rates, as reported by the CDC/SART for 2009, cite 4 to 12% live birth rates in women over age 41. Pre-treatment with DHEA fol-

lowed by IVF in our study resulted in an improved live birth rate of 21% per embryo transfer which is the ultimate marker of oocyte quality. Furthermore our results are in keeping with the small prospectively randomized controlled trial of Shulman that reported a 23% live birth rate in the DHEA group as compared with 4% in the control group (12).

The mechanism of action of DHEA is still unclear. As discussed by Gleicher in his excellent review of ovarian aging, a direct effect of DHEA in rescuing or rejuvenating aged oocytes seems unlikely (4). New research with androgen receptor knockout mice has shown that ovarian androgens are essential to folliculogenesis (15), and a more plausible concept of DHEA action may be that of follicular conditioning resulting from an improvement in the ovarian environment leading to improved oocyte quality. This may be in addition to the beneficial effect of DHEA on mitochondrial function (16). Our centre has recently added the mitochondrial nutrient coenzyme Q10 in a dose of 600 mg per day as an additional supplement for older women and others with DOR in response to the positive results from mouse studies reported by Bentov and Casper (17).

In summary, androgen supplementation with DHEA in older women represents a safe, effective, low cost therapeutic modality to improve the ovarian environment and decrease aneuploidy that leads to excellent clinical pregnancy and live birth rates with IVF in this worst prognosis group of women who have failed to conceive by other means. Although we have had babies born to DHEA-treated patients up to age 46, both spontaneously and with COH-IUI, this study and the Shulman RCT strongly suggest that women age 43 and over who have failed to conceive despite treatment with DHEA, should not be offered IVF. They should instead be counselled with regard to the greater success of donor oocyte programmes.

Acknowledgement

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Prospective evaluation of bone mass in women with gonadal dysgenesis undergoing hormone therapy – a 5-year analysis

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Introduction

Since the last two decades, it has been well-known that osteoporosis is an important public health issue. Osteoporosis is the most common osteometabolic disease responsible for high medical expenses that mainly arise from the treatment of fractures. It has been predicted that half of north american women over the age of 50 years will have osteoporosis-related fractures during their lifetime.

Adequate pubertal development with a resulting increase in hormone production has a fundamental role in bone development. It is considered the most important known factor for bone development, following the genetic factor. Thus, women with low bone mineral content due to hypoestrogenism in puberty are at increased risk for osteoporosis. Therefore, women affected by gonadal dysgenesis and consequently primary hypoestrogenism belong to a high-risk group for osteoporosis.

It is known that the administration of estrogens combined or not with progesterone, is capable of stimulating bone mass. Therefore, it is theoretically possible for the exogeneous administration of sex steroids to replace the lack of endogeneous production. However, the extent to which exogeneous hormones can replace ovarian function in sustaining bone mass remains largely unknown.

Objectives

The aim of the article is to compare bone mineral density in women with primary amenorrhea due to Turner's syndrome 45,X0 (TS) and pure gonadal dysgenesis 46,XX (PGD) at the time of diagnosis and after five years of hormone therapy (HT) use.

Methods

Twenty-nine women diagnosed with primary amenorrhea due to PGD (46, XX) and TS (45, X0) were evaluated by bone mineral density tests before HT and after five years of HT use. Women with chronic disorders or those using medication that might interfere in bone metabolism were excluded from the study. The mean age and BMI of the patients, bone mineral density (BMD) levels before and after HT use and the correlation between patient age and response to treatment were assessed. The study was conducted in the Department of Obstetrics and Gynecology of the Universidade Estadual de Campinas, UNICAMP, Medical School.

Results

The mean age of the women was 23.8 years at the beginning of treatment and 28.8 years (ranging from 17 to 38 years) in the study. The mean BMI was 23.3 kg/m^2 (ranging from 17.03 to 35.15 Kg/m^2), with little difference between women, regardless of age. Of the 29 women, concerning the initial evaluation of lumbar bone mass (L2-L4), 28 had decreased bone mineral density, 15 were osteopenic (51.7%), 13 was osteoporotic (44.8%), and 1 (3.44%) had normal bone mass. After 5 years of treatment, the only patient who already had normal BMD values, gained bone mass. Among the 15 osteopenic women, 1 (6.6%)

worsened and became osteoporotic; 6 (40%) gained bone mass and reached the normal range; and 8 (53.3%), despite showing improvement in bone mass, remained osteopenic. Concerning the 13 osteoporotic women, in 2 (15.3%) BMD was normalized, 6 (46.1%) became osteopenic, 4 (30.7%) remained osteoporotic, despite gaining bone mass and 1 (7.6%) had no alteration in BMD values. Mean T- and Z-values of bone mineral density tests in these patients were shown in Table 1 and were statistically significant. Regarding the initial assessment of bone mass at the femoral neck, of the 29 studied women, 13 (44.8%) were osteopenic, 3 (10.3%) were osteoporotic and 13 (44.8%) had normal BMD. After treatment of the osteopenic women, 3 (23.07%) had a normal BMD, 8 (61.5%) remained osteopenic but gained bone mass and 2 (15.38%) had a worse bone mass but did not become osteoporotic. Among the 3 osteoporotic women evaluated at the beginning of treatment, 2 remained osteoporotic, with 1 showing improvement in bone mass and 1 worsening, and the third patient had a normalized BMD. For the 13 patients with normal BMD at the femoral neck, 4 (30.76%) became osteopenic, 2 (15.38%) maintained bone mass and 7 (53.8%) achieved a gain in bone mass (Table 2). Mean T and Z values of bone mineral density tests in these patients were shown in Table 2 and there was no statistical significance. Regarding patient age at the beginning of treatment, a better therapeutic response was noted among the younger women.

Discussion

The proposal of this study was to take a new look at patients with gonadal dysgenesis based on the treatment of these women, treatment outcome and the relationships with independent variables such as age and BMI.

The current study retrospectively evaluated BMD in women over a period of 5 years and showed that treatment with the protocol used by the endocrine gynecology outpatient clinic is beneficial for patients and should be widely encouraged. Treatment with a regimen of estrogen and progestin showed a significant difference in the lumbar spine and the majority of women changed from an osteoporotic to normal status. In contrast, when the femoral neck was evaluated, no significant difference was found. Therefore, only BMD values of the lumbar spine were used for the remaining statistical analysis and femoral neck BMD values were rejected. This study corroborates the findings of other studies showing that treatment with estrogen and progestin, alone or in combination, may increase the BMD values found and prevent osteoporosis. When comparing estrogen alone or progestin alone regarding the best dose used, it was not possible to establish a significant difference in BMD. Women with gonadal dysgenesis should be treated with a dose regimen combining estrogen and progestin, initiating treatment as soon as possible. If feasible, treatment should be started at the time of diagnosis, since patients are diagnosed in the transition between puberty and adulthood. We could thus reproduce the peak of normal bone mass and decrease osteoporosis along with its consequences.

A linear correlation between independent variables, such as age and BMI, and BMD of the lumbar spine had different results. While a correlation between lower ages in the beginning of treatment and better BMD outcomes proved to be significant, there was no significant correlation between BMI and BMD.

BMI had no influence on lumbar spine density, although this correlation has been demonstrated in other studies. The influence of BMI and body weight is also significant for determining BMD. We believe that the results of this study were not significant between BMI and BMD because the mean BMI values of the women studied were within the normal range. Therefore, the impact of bone remodeling was limited and did not prove to be significant in this study. However, analysis was still able to show that the higher the BMI was at the beginning of treatment, the greater the gain in BMD. This confirmed the previously discussed concepts.

TABLE 1 - BONE MASS DENSITY IN THE BEGINNING AND IN THE END OF TREATMENT.

	Initial	Final	Percentage gain	P-value
Z-score lumbar spine	-2.51	-1.54	37.28%	<0.0001
T-score lumbar spine	-2.54	-1.60	33.67%	<0.0001
Z-score femoral neck	-1.11	-0.88	7.88%	0.0621
T-score femoral neck	-1.20	-0.92	22.17%	0.0769

Conclusions

Women with Turner's syndrome and gonadal dysgenesis (46, XX karyotype) undergoing long-term hormone therapy gained a significant amount of bone mass. Treatment evoked a stronger response at the lumbar spine than at the femoral neck. The importance of therapy and the need to stimulate patient adherence to treatment were highlighted. Furthermore,

the better therapeutic response among younger women underlined the importance of early diagnosis and initiation of treatment.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Management of the polycystic ovary syndrome: metformina vs pioglitazone

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Introduction

The polycystic ovary sindrome (PCOS) strikes around, 5-10% of the women in fertile age (37). Currently it is held a real plurimetabolic syndrome (1,2,3). His diagnosis often is based on the criteria of PCOS Consensus Workshop 2003, which define the presence of PCOS: oligo- and/or anovulation, clinical signs and/or biochemical hyperandrogenism, polycystic ovarian ultrasonography texture, in the absence of congenital adrenal hyperplasia, Cushing's syndrome and androgen-secreting ovarian or adrenal tumors (4,21). The substratum physiopathologic, that subtends the to establish him of the clinical alterations, biochemists and echography typical of the PCOS, is not entirely still clarified (3,38,39). However, since insulin resistance (IR) has been recognized as the most important factor in the pathogenesis of polycystic ovary syndrome, the framework of concomitant disease or result has been greatly expanded (5). It seems that (6,7,8), the IR and the consequent hyperinsulinemia, produce the excess of androgens, because of alterations in the mechanisms of phosphorylation post-receptor to peripheral level. Hyperinsulinemia has been implicated in mechanisms that increase stromal ovarian steroidogenesis and the activation of cytochrome P-450 17α · c and 3-β-hydroxy-steroid dehydrogenase in the adrenal cortex and ovary (9,40). Given the central role played by IR (14,42), in women with polycystic ovary talking about an increased risk for diseases such as type II diabetes, dyslipidemia and cardiovascular accidents (10,11,13). Thus the way is open to the use of insulin-sensitizing drugs (12). The first is metformin, which does not seem to always give satisfactory results (18,19). Instead, as part of the thiazolidinedione, pioglitazone has proved very effective, both in diabetes mellitus type II, which in PCOS (15,16,17). In the series presented here, we compare the results obtained with metformin than with pioglitazone in 240 patients with PCOS. We evaluate in detail the methods of treatment, the ability to maintain the results achieved and to prevent long-term consequences of the syndrome, with a minimal dose of medication.

Materials and methods

Recruit 240 women with PCOS. 200 with symptoms dating back to menarche (group A), and 40 from 3-4 years (group B). In group A, 160 women were not interested in conceive, aged between 18 and 24 years, 40 were female sterile or infertile, aged between 22 and 34 years old and eager to children. In group B, the age ranges were similar, and 8 were desirous of offspring. In all patients the BMI was calculated. Among the group A patients, 104 were overweight or obese. In about half were no signs of hyperandrogenism. Among the patients in group B, 16 were overweight or obese. In one third of them were no signs of hyperandrogenism. In all patients we measured: thyroid-stimulating hormone (TSH), thyroid peroxidase (TPO), Δ4 androstenedione (Δ4), prolactin (PRL), dehydroepiandrosterone free (DHEA), dehydroepiandrosterone sulfate (DHEAS), total testosterone (TT), testosterone free (FT), luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17-α-hydroxyprogesterone (17OHP). We calculated, finally, insulin and glucose. The same tests were performed in a group of 50 healthy subjects, taken as a control group. Progesterone was measured in 80 cases, in which the men-

strual cycles were irregular, the duration increased. Progesterone was measured serially every 5-6 days, until the onset of the next menstrual flow. DHEA, DHEAS, PRL, TP were assayed by immuno-chemoluminescence (CIBA Corning ACS-180), TSH, TPO, Δ4, FTP, and insulin, with radio-immunological methods (Diagnostic Products), glucose by the method of glucose oxidase (Beckman Instruments). Criteria for the assessment of insulin resistance and hyperinsulinemia were:

1) insulin resistance, calculated by HOMA-IR adjusted for BMI. According to this parameter, you can talk about IR with BMI values greater than 2.5 to 25, above 3.2 for BMI between 25 and 35, and 3.8 for BMI greater than 35 (6,41);
2) hyperinsulinemia, calculated with the area under the curve insulinemic (AUC) after oral glucose load (75 g), with sampling every 30 minutes to two hours. As a parameter we considered the normal maximum AUC obtained in control subjects. Instead, we considered indicative of hyperinsulinemia, the AUC in excess of at least 25% of those considered normal.

Identified the status of IR and hyperinsulinemia, we treated the patients as follows:

– SCHEME 1 – Overweight or obese patients = weight reduction program, with a 1200-1300 cal diet and physical activity. After 4 months we have evaluated the weight loss and normalization of both the clinical picture of Homo-IR. If the answer is satisfactory, we continued with the program, otherwise we switched to schedule 2 (which we have applied from the beginning to the patients of normal weight).

– SCHEME 2 – Patients of normal weight patients in the failed Scheme 1 = 24 patients treated with metformin, starting with 1 tablet of 500 mg daily, with increments of 500 mg every 2 weeks until the dose of 2000 mg per day. 154 patients treated with pioglitazone, with cp 15 or 30 mg, according to HOME-IR and AUC. Reevaluation of laboratory parameters at 6 months.

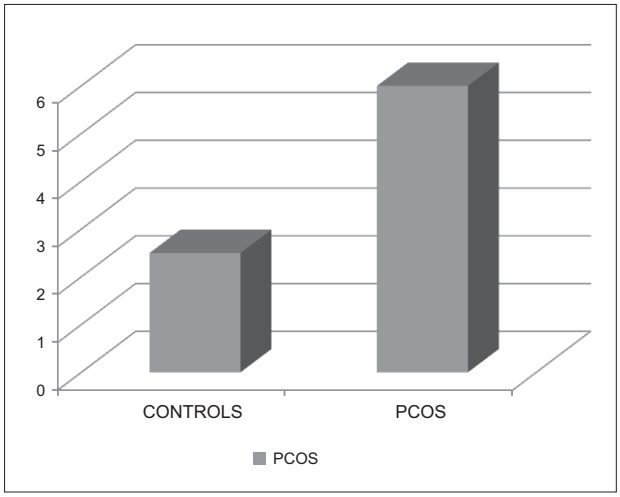
TABLE 1 - ANDROGEN LEVELS IN PATIENTS WITH PCOS (200) AND CONTROLS (50).

	PCOS	Controls	Significance
TT	0,66 +/- SD 0,15	0,57 +/- SD 0,13	NS
FT	2,12 +/- SD 0,64	1,5 +/- SD 0,48	0,05 <P <0,1
DELTA-4	2,05 +/- SD 0,53	1,74 +/- SD 0,39	0,5 <P <1
DHEA	5,1 +/- SD 1,4	4,6 +/- SD 1,25	NS
DHEA-S	303,5 +/- SD 95,7	321+/- SD 92,7	NS
17OHP	1,31 +/- SD 0,37	0,9 +/- SD 0,29	0,5 <P <1

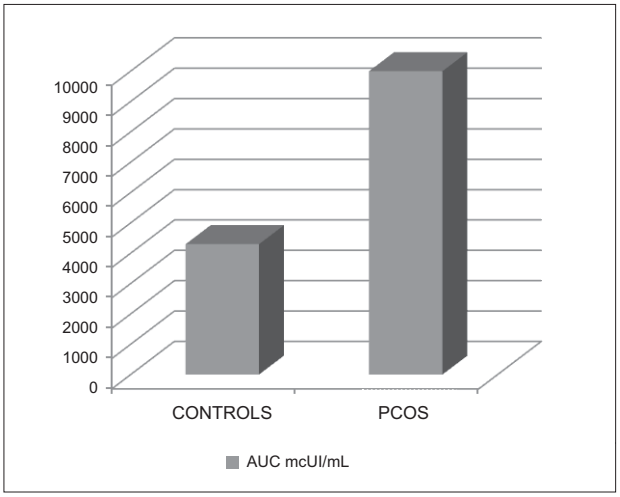
TT = total testosterone (ng/ml) v.n. 0,06-0,86
FT = free testosterone (pg/ml) v.n. 0,2-2,5
Delta - 4 androstendione (ng/ml) v.n. 0,6-3
DHEA = Deidroepiandrosterone (in ng/ml) v.n. 1,5-8
DHEAS =Deidroepiandrosterone sulfate (in mcg/dl) v.n. 120-410
17OHP = 17 idrossi-progesterone (in ng/ml) v.n. 0,5-1,7 (follicular phase)

Results

In 184 of the 200 patients in group A 'HOMA-IR and AUC were indicative of IR and hyperinsulinemia. Among the 40 patients in group B, the same parameters were affected IN16 women, all overweight or obese. The hormonal assays of patients with IR and hyperinsulinemia, compared with the controls, are in Table 1. The HOMA-IR of patients, compared with controls, are shown in Table 2. In Table 3 shows the AUC data. In 184 patients in group A with IR, 90 were overweight or obese and have therefore been subjected to Schema1, along with 16 obese patients in



Tab. 2 - Mean values of HOMA-IR in patients with polycystic ovary syndrome (PCOS) and controls (0.005<P<0.01).

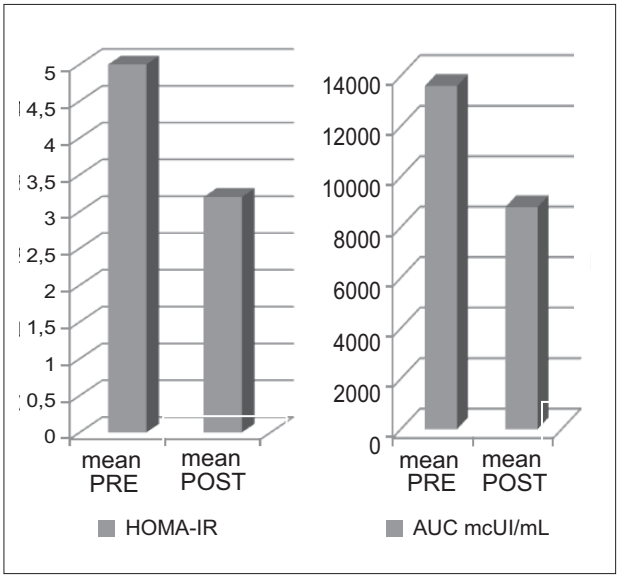


Tab. 3 - Values of the area under the curve insulinemic (AUC) in patients with PCOS and controls (0.005<p<0.01).

group B. 20 patients in group A lost weight, but only in 12 there was normalization 'HOMA-IR and AUC, with regularization cycles. These have continued to Schema1. In group B lost weight, 10 of 16 patients. Total (Group A +Group B) have lost weight, 30 (28%) patients and of these, 22 (20%) were also regulated by the metabolic and clinical point of view. It seems that the group B patients lose weight more easily. The 84 patients in whom it failed Schema1, plus 94 normal weight, were sent to Scheme 2. 24 patients were treated with metformin. Metformin was not always well tolerated: 6 patients had significant disturbances (abdominal cramps, diarrhea) with a dose of 1500 mg / die, 6 mg with that of 2000, so they had to stop at 1000 and 1500 mg/die, respectively. Only 6 patients (all obese) regularization of 24 (25%) have obtained the clinical and metabolic (Table 4). Desirous of offspring of 8, 2 gave birth to an end. From this, it is clear that only works with metformin therapy in obese patients, are not effective in 51% of patients of normal weight. The remaining 154 patients were treated with pioglitazone. 15 mg / day, in 110 patients with HOMA-IR between 2.5 and 3.5, and 30 mg / day in patients with HOMA-IR> 3.5. In 116 (75,3%) women was obtained in 3-4 months, the regularization of the menstrual cycle and the resumption of ovulation. Of the 32 patients, desirous of offspring, 18 have given birth to an end. The hormonal results evaluated after 6 months (except for TSH and TPO), are in Table 5. With regard to metabolic parameters, the comparison with baseline data, can detect a drastic reduction in both AUC home-IR, with a complete clinical and biochemical normalization in all cases (Table 6). After 4 months, we halved the dosage, while the results obtained. In 26 patients we continued with 7.5 mg / day, and 3 with 7.5 mg every other day. Pioglitazone was well tolerated and never stopped.

Discussion

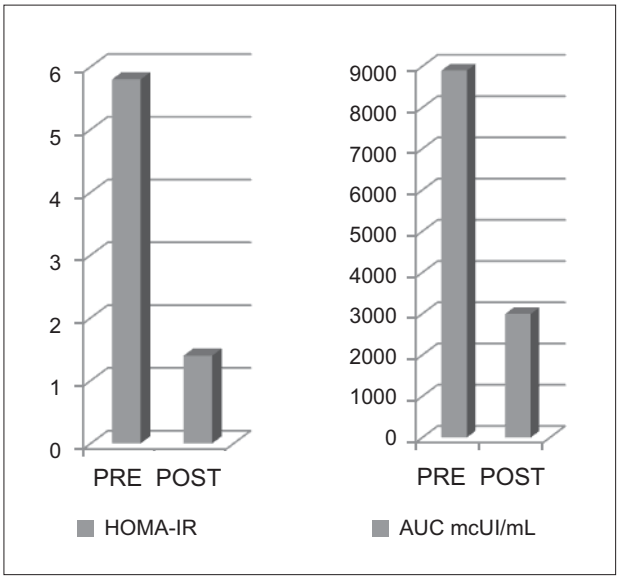
We report the high incidence of IR and hyperinsulinemia (184 of 200) patients in group A, while patients in group B, these metabolic abnormalities were present only in 16 overweight or obese. One hypothesis might be that, in this second category of patients, the IR and hyperinsulinemia do not depend on PCOS, but obesity (22,23). On this basis, it could explain why these patients seem to have less difficulty in losing weight. With regard to the results we can say that, metformin was poorly tolerated and was effective in only 25% of the patients, all of normal weight. This date does not comfort us, whereas the majority of women with PCOS are overweight or obese. Instead,



Tab. 4 - Effects of metformin treatment on HOMA-IR (0.05<p<0.1) and AUC (0.05<p<0.1) in obese patients responsive.

TABLE 5 - COMPARISON BETWEEN THE MEASURED LEVELS OF ANDROGENS IN PCOS PATIENTS BEFORE AND AFTER 4 MONTHS OF TREATMENT PIOGLITAZONE.

	PCOS Pre	PCOS Post	Significance
TT	0,66 +/- SD 0,15	0,65 +/- SD 0,16	NS
FT	2,12 +/- SD 0,64	2,16 +/- SD 0,6	NS
DELTA 4	2,05 +/- SD 0,53	1,77 +/- SD 0,48	0,05<P<0,1
DHEA	5,1 +/- SD 1,4	5,06 +/- SD 1,35	NS
DHEAS	303,5 +/- SD 95,7	321,7 +/- SD 87,4	NS
17-OHP	1,31 +/- SD 0,37	1,21 +/- SD 0,43	0,5 <P <1



Tab. 6 - Comparison of the values of HOMA-IR (0.005<p<0,01) and AUC (0.005<p0.01) in PCOS patients, before and after 4 months of treatment with pioglitazone

pioglitazone, has worked in 75.3% of cases, regardless of body weight. Moreover, it is well tolerated and does not give side effects. In conclusion, decide to continue treatment with Pioglitazone depends on several considerations (43,44):

1. In cases of chronic anovulation, as demonstrated in our patients, there is an increased risk of adenocarcinoma of the endometrium, associated with prolonged exposure to estrogens, unbalanced by progesterone production. These patients should be placed with more or less monthly rate of progesterone or a progestin (24,25). Therefore, the use of pioglitazone, should be preferred over the use of progesterone.
2. The risk of type II diabetes in patients with PCOS, IR and hyperinsulinemia, is very high (46). Predictive criteria have decreased glucose tolerance, or a positive family history of type II diabetes (14,26,29). However, given the high percentage of IR, it seems appropriate to recommend a therapy with pioglitazone, considered, that is well established its protective effect on the function of pancreatic beta-cells (27,28).
3. Cardiovascular risk factors in patients with PCOS have increased: dyslipidemia, hypertension, endothelial dysfunction, atherosclerosis (13,31,32). In many studies (33,34,35) showed that pioglitazone decreases markers considered predictive of cardiovascular risk.
4. Finally, our data prospect of being able to continue treatment even at low doses of pioglitazone, while the results over time (45).

We believe that treatment with doses of 7.5 mg on alternate days for 6 months, is far more acceptable in economic terms, and physicians, when compared with standard doses of 30 or 45 mg (17). This is even more appropriate in the light of a new update of the information security, announced June 17, 2011 by the Food and Drug Administration of Pioglitazone on the label, with reference to the increased risk of bladder cancer in patients who the taking the drug for more than one year, maximum dosage for antidiabetic therapy (36).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

How to improve user satisfaction and continuation with the new hormonal contraceptive containing nomegestrol acetate and estradiol (Zoely®)

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The new hormonal contraceptive containing nomegestrol acetate (NOMAC) and estradiol (E2) has some pharmacological advantages over the existing hormonal contraceptives. Patients will effectively be convinced and keep using this good contraceptive only if the prescriber is able to properly convince them. The main advantages of NOMAC/ E2 will be summarized first. Then some strategies to improve patient compliance will be suggested in order to better traduce a very good pharmacological profile into an effective prescription of Zoley®.

Nomegestrol advantages vs other progestogens

Nomegestrol acetate has many advantages, versus the other progestogens, because of its pure progestinic effects, longer half life and good metabolical neutrality (1).
NOMAC acetate is a potent, highly selective progestogen: a full agonist at the progesterone receptor, with no or minimal binding to other steroid receptors, including the androgen and glucocorticoid receptors. It demonstrates moderate antiandrogenic activity and strong antiestrogenic activity in animal models. NOMAC is associated with effective suppression of gonadotropic activity and ovulation in premenopausal women, and a neutral impact on hemostasis, lipids, and carbohydrate metabolism. It has shown favorable effects on estrogen metabolism, in normal and cancerous human breast tissue. In human breast cancer cell lines in vitro, it does not stimulate cell proliferation. NOMAC in combination with E2 has a good cycle control and a favorable safety profile.
The monophasic NOMAC 2.5 mg and 17β-estradiol (E2) 1.5mgc contraceptive administered in a 24/4-day regimen provides a consistent and robust ovulation inhibition. Zoely® provides high contraceptive efficacy with acceptable cycle control as well as an overall adverse event profile similar to that of drospirenone/EE pills (2). There is a less overall impact with NOMAC/E2 on hemostatic, lipid, inflammatory, and carbohydrate metabolism parameters than with levonorgestrel 150 µg/EE 30 µg (3). These clinical findings are promising even though the Cochrane database has no jet data to demonstrate differences between NOMAC and the existing other progestogens used for contraception. (4)

Estradiol advantages vs etinilestradiol

Natural estradiol (E2) has many benefits, versus etinilestradiol (EE), for metabolism and coagulation, but also a potential benefit for sexuality.
Lowering the estrogen dose and potency is a long term evolution strategy that makes hormonal contraception safer, even though it could cause more intermenstrual bleeding (5).
NOMAC/E2 could have a more favourable venous thromboembolism risk profile than levonorgestrel/EE, also for the lower liver effects of E2, even though further epidemiological data are required to confirm this (6).
E2 seems to have a better estrogenic vaginal effect than the 20 mcg of EE that could cause more vaginal dryness. The lower effect on SHBG could make Zoely® more neutral as to the effect on the androgenic mechanisms of sexual desire.

Natural E2 could be more acceptable versus EE, being the physiologic estrogen.
The 24-day NOMAC/E(2) regimen is associated with greater inhibition of follicular growth and shorter duration of withdrawal bleeding than the 21-day regimen used by most of theexisting contraceptives. The shorter pill-free interval results in a greater margin of contraceptive efficacy and tolerability and fewer withdrawal symptoms (7).

Enhancing compliance to the NOMAC/E2 contraceptive (Zoely®)

It is not easy to explain to the patients the reasons to preferentially prescribe this new contraceptive using short, easy to understand and effective sentences, but the way it is presented will strongly affect its acceptance and continuation.
An improvement of the doctor patient relationship and some communication strategies summarized here will help the prescriber to improve Zoely® compliance.

1. **Find a proper setting and enough time for the first prescription.**
   The first prescription is an important moment for its acceptance and continuation. Try to do that in a place and in a moment when a patients can express her needs and concerns and the doctor can address that, having at least few minutes of communication without being interrupted. This is true for every prescription but making the patient be really convinced about the choice of Zoely® needs that.
2. **Create a good doctor-patient relationship to make the patient trust the doctor.**
   The patient has to perceive that the doctor is really interested in what she expresses and wants the best for her. The strategies to do that are difficult to teach. Most of them are based on non verbal communication, could be instinctive and for the majority unconscious. Some women will experience mild initial side effects. Those who trust their doctor will trust his/her prescription and do not stop it.
3. **Inquire which are the most important reasons to use, and to fear, a hormonal contraceptive for that specific women.**
   Find out which are the main reasons for using or stop taking a contraceptive for each women helps to focus the discussion on the main relevant arguments. Zoley® has a lot of advantages to use to convince the patient to choose it. They are summarized above. The benefits of NOMAC and the natural and neutral effects of E2 are the main subjects to use.
4. **Try to understand if there are hidden questions that could lie behind what the women expresses.**
   Many times the "real" reason why a contraceptive is needed, or particularly feared, are not openly expressed or even conscious to the patient. If the doctor feels a "resistence" beyond rational reasons this could be a sign that there is something hidden, to find out and discuss, that prevents the women to use the prescription.
5. **Use the proper way to communicate according to the patient needs.**
   The ideal doctor patient relation is "adult to adult": a peer to peer discussion of the prescription of Zoely®. Some women prefer a "parent to children" way of relation and let the doctor decide for them. They like and need more reassurance than information on the pharmacological profile of NOMAC/E2.
6. **Discuss relevant arguments understandable for the patient in an easy to remember way.**
   The discussion should be short and effective so it is important to discuss only the relevant arguments, restricted to only few subjects (one to three). The reasons of the choice of a NOMAC/E2 should use arguments relevant to the needs of that specific patient. The way to explain them has to be as simple and easy to keep as possible.
7. **Anticipate openly the possibility of adverse effects and what to do if it happens.**
   Some patients will have side effects even with a very well tolerated contraceptive like Zoely®. An open discussion to inform about some of them, like the occurrence abnormal bleeding, is better than letting the women discover or experience that later. The women that know what to do keep using the pill end wait for the likely spontaneous disappearance of the side affects after the first months of use.
8. **Reassure using consciously the non verbal communication.**
   Even if a women is rationally convinced to use NOMAC/E2 some unconscious fears may persist. The doctor should be aware that his/her non verbal communication can strongly strengthen or reduce the good arguments used. If, for example, the doctor is worried about his/her own problems and expresses that with the mimic or the voice, the women feels that and tends to be more scared about using the contraceptive prescribed.
9. **Try to be available in case of doubts or side effects occurrence.**
   If the women reads scaring information on the package leflet or she will experince some side effects, like bleeding or amenorrhea, the possibility

to have an easily available, even only telephonic, reassurance at the time of occurrence prevents discontinuation of Zoely®.

10. **Follow up in a short time and then periodically.** The first months of use are the most important for the continuation. Generally side effects appear in this period and most of them disappear spontaneously. An appointment within few months could help to discuss any new event and to help the women keep using the NOMAC/E2 contraceptive with all its benefits versus the other contraceptives. A further periodical check could help evaluating any new need, symptom or sign and make this good contraceptive use even safer.

Conclusion

The NOMAC/E2 contraceptive has some advantages over the existing contraceptives, but they should be properly explained. In order to obtain the best compliance the prescriber not only have to tell the women the pharmacologic advantages of Zolely®: he/she should better express them by using some clinical communication strategies.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Prospective study of 162 deliveries with vacuum extraction (VE)

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Objective

Determining the perinatal events and the associated material to the delivery with the help of VE.

Methodology

We prospectively studied 162 where delivery's have been conducted with the help of VE in the period January-December 2009.

Results

Of 162 cases, 33% of cases have been nulipara, and 66% multipara. VE delivery's are carried out in 97% of cases, while the S.C in 2% of cases.
VE is applied <10 min in 97.4% with a traksion.
Four cases or 2.5% of cases have manifested signs of bleeding in the newborn intracranial where three cases or 1.8% have no neurological deficit manifested signs regarding clinical aspects.

Conclusion

Applying calottes the head of the newborn patients where birth is conducted with the help of VE does not cause a head trauma in newborns.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Effects of hyperprolactinemia on the murine uterine prolactin gene and prolactin receptor expression

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Introduction

Hyperprolactinemia is characterized by persistently high prolactin (PRL) levels in the blood, and it is the most common endocrine disorder of pituitary hypersecretion on the hypothalamic-pituitary axis (1,2).
The treatment of choice for hyperprolactinemia is dopamine agonist administration, which inhibits PRL synthesis and release. Conversely, a dopamine antagonist may raise serum PRL levels, leading to hyperprolactinemia. In fact, it has been shown that metoclopramide is efficient in the development of a hyperprolactinemia model (3,4).
Metoclopramide-induced hyperprolactinemia in female mice (5) reduces the synthesis of not only ovarian steroids during proestrus but also progesterone during pregnancy. The concentration and gene expression of PRL receptors are affected as well given their dependence on the serum levels of sex hormones (6).
The role of hyperprolactinemia interaction with sex steroids in the gene expression of PRL and its receptors in the uterus is still controversial. We thus undertook this study to evaluate the gene expression of PRL and its receptors in the uterus after sex steroid administration using the real-time PCR technique.

Materials and methods

This research was approved by the local institutional committee.
Of 49 female mice (SWISS) with a normal estrous cycle, previously checked by vaginal smears taken for 7 days, a random (13) total of 35 underwent bilateral oophorectomy and were then confined to cages for 20 days. After this postoperative adjustment period, these animals were randomly allocated (13) to 5 groups, and the nonoophorectomized mice to 2 groups. The groups, all of equal size (n=7), were GI (nonoophorectomized control mice), GII (nonoophorectomized mice with untreated hyperprolactinemia), GIII (oophorectomized control mice), GIV (oophorectomized mice with untreated hyperprolactinemia), GV (oophorectomized mice with hyperprolactinemia treated with estrogen), GVI (oophorectomized mice with hyperprolactinemia treated with progestogen), and GVII (oophorectomized mice with hyperprolactinemia treated with estrogen and progestogen).
The drugs, which were administered subcutaneously for 50 consecutive days, were as follows: metoclopramide (Sigma®), 200μg/day in 0.9% saline solution; 17β-estradiol (Sigma®) and micronized progesterone (Sigma®), 1μg/day and 1mg/day, respectively, in oil-based solution (3,4). On the last day, one hour after drug administration, the mice were sacrificed, except for those not in proestrus. These continued to receive the drugs until they entered that phase (1).

Sample collection and processing
The mice were killed by decapitation and their left and right uterine horns were removed for total RNA extraction through TRIzol® (Invitrogen). The RNA thus obtained was resuspended in sterile MilliQ water and stored in a freezer at -80° C.

Qualitative RNA analysis, cDNA (complementary DNA) synthesis, and quantitative real-time PCR (qRT-PCR)
Total RNA was treated with DNase I (Invitrogen) to remove any genomic DNA contamination. Subse-

quent synthesis of cDNA was performed using up to 2µg of total RNA and the Hi Capacity Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer`s instructions.

Qualitative cDNA analysis
The cDNAs were analyzed by the conventional endpoint PCR technique, using the pairs of specific activators for the β-actin gene. Following interpretation of cDNA quality in agarose gels (Sigma®), the cDNAs were utilized in the qRT-PCR reactions (14).
Simultaneously, standardization of reactions with the specific initiators for the 4 prolactin receptor (PRL-R) isoforms, L, S1, S2, and S3, was carried out. Endometrial prolactin expression was also assessed.

Statistical analysis
The results were subjected to the analysis of variance (ANOVA) and, additionally when necessary, to the Tukey-Kramer multiple comparisons test to check for significant differences between the experimental groups. The significance level was set at 5% ($p<0.05$).

Results

Gene expression analysis of endometrial prolactin and its receptors
As shown by transcript analysis for endometrial prolactin, expression of such a gene was highest in GIV and lowest in GIII ($p<0.05$).
Expression of the long endometrial prolactin receptor isoform was highest in GVI and GVII and lowest in GI and GIII ($p<0.05$). Of the short prolactin receptor isoforms, PS1, PS2, and PS3 alone were evaluated. The highest expression of PS1 was found in GIII, and the lowest, in GIV and GVI ($p<0.05$). Expression of PS2 was highest in GII and GIII and lowest in GVI and GVII ($p<0.05$). PS3 was mostly expressed in GVII and least so in GIII ($p<0.05$).

Discussion and conclusion

Prolactin is known to interact with estroprogestogen therapy (7), and such knowledge was further confirmed by this study. Our results highlight the influence of progesterone in increasing gene expression for the PRL oligonucleotide while estrogen action remains at a low point. Despite such an effect, when progesterone is administered along with estrogen, gene expression decreases. Thus, estrogen therapy, as well as estroprogestogen therapy, may benefit the uterus as regards prolactin expression in a hyperprolactinemic state.
Understanding prolactin receptor behavior is another relevant aspect targeted in the past two decades (8). Yet, little is known about prolactin receptor interaction in animals subjected to a hyperprolactinemic state.
Expression analysis of the receptors showed that all isoforms (PL, PS1, PS2, and PS3) were expressed in all of the study groups. This underscores the sensitivity of the technique that was chosen (9). In contrast, Rossi et al. (2009) was not able to detect transcripts for endometrial prolactin receptors in any of the oophorectomized animals with hyperprolactinemia. These divergent results appear to stem from the choice of the analytical techniques employed.
PRL receptors are expressed in diverse organs, such as the uterus and the hypophysis (10). However, their concentrations are known to be dependent on sex hormone levels in the blood (4,6) as corroborated by our results that showed that the higher the serum concentration of these steroids, the higher the expression of the long isoforms of the receptor.
Sex steroids are fundamental in establishing the expression levels of the prolactin receptor (11,12). In the present study, such an effect was manifested by the increased expression of the long receptor isoforms, and this took place whether the hormones were administered separately (GV and GVI) or conjointly (GVII).

The authors report no conflict of interests.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Hyperandrogenism with women smokers and its role in the prediction of smoking cessation treatment success

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Introduction

Smoking represents the most widespread substance dependence in the world. The negative impact of tobacco smoking is complex. However, it influences biosynthesis and steroids effects among others. Smoking interferes with neuroactive steroid metabolism and addiction arises very quickly.

Smoking in women has an anti-estrogenic effect. It may also influence hormonal contraception effects negatively: this may lead to higher incidence of off-cycle spotting and bleeding (Rosenberg et al. Am J Obstet Gynecol 1996).

However, anti-estrogenic effect has not been confirmed in all studies. On the contrary, the increase in androgen production in women smokers throughout life from puberty to menopause has been described in studies consistently (Van Voorhis et al. Fertil Steril. 1992; Barbieri et al. Fertil Steril. 2005; Manjer et al. Eur J Epidemiol. 2005; Cochran et al. Obstet Gynecol. 2008; Cupisti et al. Fertil Steril. 2010). A number of studies have focused on the impact of smoking on female reproduction. They have reported increased incidence of infertility in women smokers (Thomford and Mattison, J Ark Med Soc 1986; Weigert et al. J Assist Reprod Genet 1999).

Patients and methods

The study involved 40 premenopausal and 60 postmenopausal women heavy smokers who had decided to stop smoking and sought medical help at the Centre for Tobacco Dependence. The women did not use hormonal contraception or hormonal replacement therapy for at least 6 months before the testing, they did not suffer from any serious illness and did not use any medication affecting steroidogenesis. The premenopausal women had regular menstrual cycles. The women were examined before the initiation of smoking cessation, after 6, 12, 24 and 48 weeks of abstinence. During each examination, blood was collected to determine steroid spectrum, LH, FSH, SHBG and basic anthropometric data were measured. For evidence of abstinence from smoking, cotinine analysis was used. The study has been approved by the ethics committee of the Institute of Endocrinology.

Most of the steroids and their polar conjugates were measured using the previously described GC-MS method (Hill et al. J Steroid Biochem Mol Biol 2010). The 17-hydroxy-pregnenolone was measured by RIA as described in our previous report (Hill et al. Steroids 1999) and conjugated 17-hydroxy-pregnenolone was measured using the same method after hydrolysis as described previously. Estradiol was measured by RIA kit from Orion, Finland (intra-assay CV=4.4%, inter-assay CV=4.6%) and 17-hydroxy-progesterone was assayed by kit from Immunotech, France (intra-assay CV=5.2%, inter-assay CV=6.5%). LH by IRMA kit from Immunotech, France (intra-assay CV=3.7%, inter-assay CV=4.3%), FSH by IRMA kit from Immunotech, France (intra-assay CV=2.6%, inter-assay CV=4.5%) and SHBG by IRMA kit from Orion, Finland (intra-assay CV=6.1%, inter-assay CV=7.9%.)

The hormonal profiles were evaluated using repeated measures ANOVA model. The statistical software Statgraphics Centurion version XVI from Statpoint Inc. (Warrenton, Virginia, USA) was used for simultaneous data transformations, ANOVA testing and multiple comparisons.

Results

In the group of postmenopausal women, nine women were successful in smoking cessation, 15 women failed during the treatment, 36 women had only basal sampling. Therefore the data from postmenopausal women could be analyzed after one year abstinence. In the group of premenopausal women, only 5 women were successful, 12 women failed during the treatment and 23 women had only basal sampling. Due to the small amount of successful women, these data could be analyzed only after the first 6 week period of cessation.

In the group of premenopausal women who discontinued smoking for 6 weeks we monitored changes in C21 steroid levels on which the smoking discontinuation had no effect. We also monitored the C19 steroid levels where we found an increase in conjugated androstenediol, 5- androstene-3β, 7α, 17β-triol and decrease in conjugated androsterone and conjugated 5α-androstane-3β, 17β-diol. Changes in the levels of other androgens were not significant.

In the group of postmenopausal women, we found increasing levels of androgens (testosterone, androsterone) during smoking cessation, increasing of DHEA was insignificant. Conjugated to nonconjugated 20α- pregnanolon ratio was significantly decreasing. Changes in the levels of other C21 steroids were not significant.

The higher androgen levels before the smoking cessation correlated with failure in smoking cessation in both groups (premenopausal and postmenopausal women).

Conclusion

Smoking causes higher androgen levels in women. Our results indicate that smoking discontinuation leads to their further increase. There are several hypotheses explaining elevated levels of androgens after smoking cessation. We considered the influence of nicotine in nicotine replacement therapy. However levels of kotinin, which reflects nicotine income, do not correlate with levels of androgens. Neither gaining weight nor ageing process had influence on androgen levels. A question remains, whether testosterone itself could play a role in development of tobacco addiction. From the literature, higher levels of testosterone in female smoker's daughters are an independent risk factor for development of nicotine dependence in future (Kandel et al. Am J Public Health 1999). Smokers may therefore have higher testosterone levels than non-smokers before starting smoking. We believe that, these levels are subsequently reduced by smoking and after cessation returned to its original concentrations. Another explanation for higher levels of testosterone is irreversibly affected steroidogenesis caused by chronic smoking.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Psychobiological stress reactivity during healthy pregnancy in humans

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Research on stress provocation in pregnant women has resulted in inhomogeneous findings regarding the alterations of the hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) with respect to the ongoing pregnancy and the type of stressor. While some studies report sig. psychobiological responses to various stressors (1-4) others do not (5-7). Gaining a deeper understanding of the stress response during pregnancy is essential, as prenatal psychological stress has been linked with a range of adverse health consequences for the pregnant woman and her unborn such as an increased risk of pregnancy loss, preterm delivery and low birth weight (8,9). In a series of studies we examined the endocrine and autonomic responses (a) to standardized psychosocial stress at different stages of pregnancy and (b) to an invasive diagnostic procedure (amniocentesis, AC) during the 2nd trimester.

In study 1, we exposed 30 healthy pregnant women at the beginning of the 2nd trimester (group 1), 30 healthy pregnant women at the beginning of the 3rd trimester (group 2) and 30 healthy non-pregnant controls in the follicular phase of their menstrual cycle (group 3) to a standardized psychosocial stress test. In study 2, we monitored 34 healthy pregnant women undergoing AC for karyotyping in the 2nd trimester and re-examined them in a rest condition (RC) after they had received the inconspicuous AC test results. The stress responses to the psychosocial stress test and to the psychological challenge of the AC procedure were measured by the endocrine parameters cortisol (F) and alpha-amylase (AA) from saliva samples as well as by continuous monitoring of cardiac activity in order to obtain heart rate (HR) and heart rate variability (HRV) measures. In study 2, F and its inactive metabolite, cortisone (E), as well as a ratio between the two compounds (E/(E+F)) were additionally assessed from amniotic fluid samples. This ratio served as a proxy for the activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), an enzyme that is present in the placenta and the fetal system and inactivates F to E. 11β-HSD2 is, therefore, assumed to protect the foetus from overexposure to maternal glucocorticoids (10).

In study 1 stimulated HR and HPA responses showed sig. increases in all three groups following stress exposure. F and HR increases of both pregnant groups were shown to be comparable with those of the controls. AA increases of both pregnant groups were markedly attenuated compared to non-pregnant women. Interestingly, F recovery following the stress test was sig. prolonged in 2nd trimester pregnant women (3). This finding may be interpreted as a specific phase of stress vulnerability during pregnancy.

In study 2 AC provoked a sig. ANS rise since AA and HR increased sig. over time as compared to the RC. With regard to HRV, the high frequency component, prior to and after AC, was comparable to levels during the RC. However during the AC condition, a sig. increase over time was apparent in the period after the AC as compared to the RC. Compared to the RC, no sig. increase over time was revealed for the low frequency component of HRV, even though these levels were generally sig. higher during most of the amniocentesis condition than during the rest condition. Finally, the ratio between the low frequency and high frequency components of HRV (LF/HF) which is proposed to reflect the autonomic balance between the sympathetic and parasympathetic activity, decreased sig. over time during AC compared to RC. We subsequently examined whether the stress response of the ANS parameters were associated with the E/(E+F) ratio in the amniotic fluid. Indeed, baseline LF/HF values were negatively correlated with the E/(E+F) ratio

and positively with F in the amniotic fluid, whereas a stronger stress response of the LF/HF ratio was positively related to the E/(E+F) ratio and negatively to F in the amniotic fluid (11).

Our data provide evidence that healthy pregnant women show characteristic stress responses during pregnancy across different stressor types. Besides the use of standardized psychosocial stress tests, diagnostic medical procedures such as an AC may also serve as a useful method to examine the stress response under more real-life conditions. Our data also emphasize the importance of measuring the responses of both the HPA axis and the ANS across different stages of pregnancy. To date few studies have examined the response of AA and HRV in response to acute stress during pregnancy and our results contribute to the literature in this regard.

Furthermore, our findings indicate that allostatic processes seem to be initiated in the fetal system to counterbalance the effects of acute stress. The activity of 11β-HSD2 in response to acute psychological stress has not previously been investigated in humans. The association between a higher acute LF/HF stress response accompanied by an increased E/(E+F) ratio but with decreased F levels suggests that 11β-HSD2 activity was up-regulated in the fetoplacental unit. Even though we relied on an indirect marker of the enzyme activity and were restricted to a single measurement of amniotic fluid levels of F, E, and the E/(E+F) ratio, the results nevertheless indicate that the maternal and fetal systems are linked during the experience of acute stress.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Efficacy and acceptability of a new non-hormonal mucoadhesive vaginal moisturizing gel for the management of vaginal atrophy

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Introduction

Vaginal atrophy can occur in women of any age, although it is more prevalentduring menopause as a consequence of the decline in the endogenous estrogen production that occurs during that stage of woman's life. An estimated 10% to 40% of postmenopausal womenhave symptoms related to vaginal atrophy. Most common symptoms include vaginal dryness, vulvovaginal irritation, itching and dyspareunia, all of them causing discomfort that, if untreated, impacts significantly on woman's quality of life. Vaginal moisturizers are used on a chronic maintenance basis to replace normal vaginal secretions and are useful for vaginal symptomsrelief. Its regular use is recommended as first line therapy, especially in women wishing to avoid hormonal therapy or with a contraindication to estrogen use. The efficacy and acceptability of a new non-hormonalvaginal gel was evaluated in postmenopausal women with vaginal atrophy.

Material and methods

Fifty three postmenopausal women with symptoms of vaginal atrophy were studied. A new non-hormonal vaginal gel was used. The bioadhesive molecules in its composition adhere to the vaginal wall and thus facilitate a prolonged moisturizing and lubricating action of the glycerol present in the formulation. Each patient received 1g of the gel daily for 3 weeks and then twice weekly up to 12 weeks. The presence of symptoms of vaginal atrophy (vulvovaginal dryness, dyspareunia, burning or itching) and its intensity (graded on a scale from 0 to 3 (0=absence, 1=mild intensity, 2=moderate intensity and 3=severe intensity) was assessed by patients at baseline and after 3 and 12 weeks of treatment. Improvement of symptoms (defined as decreasing the grade of intensity of the symptom) was evaluated after 3 and 12 weeks administration. Additionally, at the end of the study women were asked to rate as excellent, good, acceptable, bad or unacceptable the acceptability of the product regarding aspects such as easiness of administration, leakage, staining or cleanliness.

Results

A total of 53 postmenopausal women, with a mean age of 57.2 years old, were enrolled in the study. Patient demographics and baseline characteristics are presented in table 1. At baseline,100% of patients pre-

TABLE 1 - DEMOGRAPHIC AND BASELINE CHARACTERISTICS.

	Vaginal moisturizing gel (n=53)
Age [mean (SD)] (years)	57.2 (6.70)
Race White [n (%)]	53 (100)
Body Mass Index [mean (SD)] (Kg/m2)	26.1 (4.52)
Time since last menstrual period [mean (SD)] (years)	10.2 (6.68)
Previous hysterectomy [n (%)]	5 (9.4)
Vaginal symptoms: [n (%)]	
- Vaginal dryness	53 (100)
- Dyspareunia	48 (90)
- Itching	29 (54.7)
- Burning	18 (34)

sented vaginal dryness, 90.5% dyspareunia, 54.7% itching and 34% burning. After 12 weeks administration an improvement of vaginal dryness, dyspareunia, itching and burningwas observed in 66.7%, 75%, 82% and 71% of women that suffered from those symptoms at baseline, respectively (Fig. 1). The most bothersome symptom also improved after 12 weeks in 74.5% of women. The mean magnitude of changeinthe intensity of the symptoms from baseline to week 12 (Fig. 2) was 1.2 for vaginal dryness ($p<0.0001$); 1.4 for dyspareunia ($p<0.0001$); 1.2 for itching ($p<0.0001$)and 1 for burning ($p=0.001$). After 3 weeks daily administration an improvement of vaginal dryness, dyspareunia, itching and burning was observed in 70.6%, 68.9%, 64% and 66% of patients that suffered from those symptoms at baseline, respectively. The mean magnitude of change in the intensity of the symptoms from baseline to week 3 (Fig. 2) was 1.0 for vaginal dryness ($p<0.0001$); 1.1 for dyspareunia ($p<0.0001$); 0.9 for itching ($p<0.001$) and 0.9 for burning ($p<0.01$). Regarding the product acceptability, 84% of patients rated the product as excellent or good in relation to ease of administration and cleaning the product, leaving no stains or residue after application. No patient considered the product as bad or unacceptable.

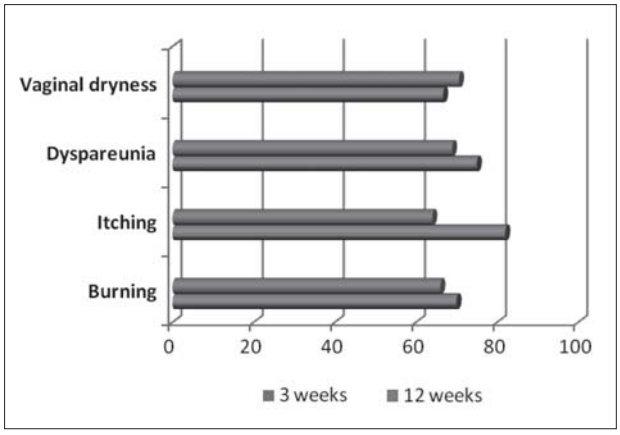

Fig. 1 - Improvement of vaginal symptoms after 3 and 12 weeks treatment: percentage of improved women (%).

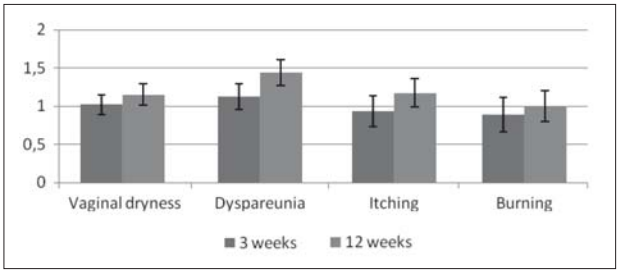

Fig. 2 - Improvement of vaginal symptoms after 3 and 12 weeks treatment: change in the intensity of the symptoms (mean±SEM).

Conclusions

Symptoms resulting from vaginal atrophy are frequent complaints of postmenopausal women.One of the primary goals forvaginal atrophy management is the symptoms relief so that women's discomfort and quality of life may be improved. Non-hormonal vaginal moisturizers are recommended as first-line therapyfor vaginal atrophy and they can be safely used by women who do not want to use hormonal options.

This study has demonstrated that a new non hormonal vaginal gel with moisturizing and lubricant properties significantly improves symptoms of vaginal atrophy, such as vaginal dryness, dyspareunia, itching and burning. Importantly, this improvement becomes evident as early as after 3 weeks of daily administration. The acceptability of the new formulation is highly favorable, as up to 85% of women consider the treatment as excellent or good.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Evaluating knowledge and attitude of high school girls towards reproductive health in Bandar-Abbas, Iran

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Introduction

Increasing emphaisis on the health of mothers, youth and birth control as main aim of control programms caused a range of health and medical activities to be developed in recent years. Moreover, the term Reproductive Health is used insead of birth control. The importance of Reproductive Health is an individuals approval as the Reproductive Right, which is part of Human Rights 1-2 According to the International Conference of Population development, Cairo 1994, the definition of Reproductive Health is state of complete physical, mental and social well-being not merly absence of disease or infirmity. It includes all aspects of the Reproductive system, process and its function, thus it means people should be able to make decision for the time, process, and alternant of of pregnancy by themselves, consciously and freely. However the sexuality and pregnancy status of youth is one of the best known aspects of our community, whereas the physical, mental and social health of communities is threatening by unsafe abortion, AIDS and their mortality. The most recent surveys show that 60 people are HIV-infected all around the world and 6 people are getting afflicted by HIV every minute. Half of the newly annual HIV-infected ones and one third of the people who take STD (Sexual Transmitted Diseas) are younger than 25 years old (1-2-3). Although the public access (spiecially for the youth) to the adequate information, facilities and having the consulting services including Reproductive Health are emphasized; there are many requirements that have not been met. As the studies show, major obstacles contain using no contraception equipment for women, inadequate consultation, lack of facilities and personnel of the Reproductive Health services (1-4-5). Since accessibility to the high level of standards and sufficient information about sexual issues and Reproductive Health is one of the major parameters of the Reproductive Health Rights, Ministray of Health and Medical sciences of Iran insists on Reproductive Health training for all ages (specially for youth) in order to solve the economic difficulties and to promote the birth control. Currently it is the responsibility of health officials and researchers, to recognize issues and to the overall interventions for preventing the goals of this study is to evaluate knowladge and attitudes of the female students about the Reproductive Health in order to suggest properly, considering the results of this study, to promote Reproductive Health in these two fields.

Aims

Main goal of the project is determing the knowlege and attitudes of female high school students about Reproductive Health in Bandar Abass-Iran 2008.

Sub-goals

Determing the knowlege of female high school students about Reproductive Health in Bandar Abass-Iran 2008. Determing the knowlege of female high school students of Bandar Abass about Reproductive Health depending on marital status. Determing the knowlege of female high school students of Bandar Abass about Reproductive Health depending on field of education. Determing the knowlege of female high school students about Reproductive Health Depending on type of school (public or private). Determing the knowlege of female high school students about Re-

productive Health depending on age. Determing the attitudes of female high school students of Bandar Abass about Reproductive Health. Determing attitudes of female students of Bandar Abases about marital status. Determing the attitudes of female high school students of Bandar Abass about Reproductive Health. Determing the attitudes of female high school students of Bandar Abass about marital status depending on field of education Determing the attitudes of female high school students of Bandar Abass about marrital status depending on type of school.

Materials and methods

In this analytical descriptive and cross-sectional study, 1200 female high school student of Bandar Abass-Iran, who were selected by cluster sampling techniques, have been studied. A well-prepared questionaire was applied which was extracted from scientific refrences and it has the scientific approval. The above- mentioned questionnaire contains 8 questions, open questions related to the demographic information which include: age, course, type of school and marital status. 8 out of 18 questions, are about Reproductive Health and 5 view points in opposition with that. The range of scores based on strongly disagree, slightly disagree, natural, slightly agree and strongly agree were 1 to 5. After collection the information questionnaire, by usig a descriptive statistical test as well as SPSS softwere version 15, the data analysed and classified. Results: we have studied 1200 female high school student in Bandar Abass-Iran among whom 180 (15%) students were bachelors. (50.4%) of them had acquaintance with the term of Reproductive Health and 49.6% of them did not have. 40.8% believed that they know how to prevent pregnancy at the time of emergency and 42.1% believed they know when Ferrus medicines should be used accurately. 510 students thought that the most common way of HIV transmission in Iran is the shared syringes and 115 students considered blood derivatives as most common reason. 42.1% believed that best method of contraception is to use condom and 38.8% considered TL (tubal ligation) as best method. 81.2% (975) students notified their familiarity to gonorrhea, and 50 students to syphilis. 60% believed that being adhered to ethical principle is the best way to prevent getting STD and 20.8% considered not to use contaminated razors or needles as best way. Only 19.2% said they know when is the accurate time for BSE (Breast Self-Examination) and 13.3% believed it is not required at all. 35% strongly agreed to delayed sexual activity until the time of marriage in order to prevent getting STD or unwanted pregnancies. 42.1% storongly disagreed that contraceptive equipment should be accessible for the youth. 59.6% strongle agreed to get familiar, for the youth, to contraceptive equipment before the time of marriage. Most of students agreed to acquaint with reproductive health and how to prevent STD or unwanted pregnancy.

Discussion

Results showed that 49.6% were not familiar to term of Reproductive Health, while others seemed not to realize different aspects of Reproductive health properly. This study confirms that students did not realize the accurate time for BSE, using Ferrous medicines during pregnancy, and lack of knowledge how to prevent pregnancy at the time of emergency.

Conclusion

These results complied with the previous studies, adhering to ethical principle, delying sexual activity until marriage time, keeping contraceptive and sexual equipment out of the youth reach and cultural status of Iran, it was expectable, as a matter of fact and it was confirmed quite significantly in the other studies all around the country. This study and other similar studies showed that vas majority of individuals accepted that different Reproductive Health training should be taken necessary for the public especially for the youth Paying attention to the training for the various age ranges of all community individuals seem to be intransitive and necessary, considering the lack of awareness about the sufficient information regarding the term Reproductive Health or subsequent complication of lacking awareness. Thus, community health responsibles, as the health providers, by using broadcasting and health announcements, play a crucial role to promote Reproductive Health.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Anti-progestin effects of selective progesterone receptor modulators (SPRM) and SPRM-like chemicals in human endometrium

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Introduction

A receptive endometrium is of crucial importance for successful embryo implantation. The human endometrium, which is unique among adult tissues, undergoes complex dynamic changes during each menstrual cycle, hosts the embryo implantation process and has been recognized as important fertility-determining factor (1). The endometrial progesterone receptor (PR) plays a crucial role in these processes and is an important target for endogenous hormones and drugs. PR antagonists and selective progesterone receptor modulators (SPRM) exert specific receptor-mediated effects on the human endometrium, and two of them (mifepristone, RU486; ulipristal acetate) are approved for emergency contraception and abort induction (2-4). Remarkably, also environmental chemicals (e.g. DDT, 4-nonylphenol, bisphenol A) and natural compounds (e.g. apigenin) can interact with the PR (5-6) as demonstrated in yeast transactivation assays. Further, numerous traditional plant preparations have an anti-implantation activity with unknown mode of action (e.g. 7). Based on these findings and in view of the rapidly growing world production of chemicals (8) the human endometrium has to be addressed as relevant target tissue for endocrine disrupting chemicals (EDC) (9). Since embryo implantation in humans is different to animals this chemical risk can only insufficiently be addressed by animal tests. New human *in vitro* models are needed to assess endometrial effects for chemical-safety testing and drug development. To our knowledge, there is currently no routine *in vitro* test available for endometrium-specific effects of antigestagenic compounds in humans. We developed a tissue-specific *in vitro* assay based on the human endometrial epithelial Ishikawa cell line to analyze the effects of antiprogestin compounds on pre-selected gene expression biomarkers.

Methods

Our recently developed *in vitro* Ishikawa model for estrogenic compounds (10) was modified for the detection of antiprogestin effects. Cells were grown for 3 days to subconfluency and primed with 17β-estradiol to up-regulate the progesterone receptor. In the test phase cells were incubated with combinations of progesterone (10^{-7} M) and variable concentrations of test compounds for 48 hrs. Reverse transcription quantitative real-time PCR (RT-qPCR) with assays from the Universal Probe Library (UPL, Roche) was applied to characterize antiprogestin effects on pre-selected gene expression biomarkers, which were identified by microarray analysis (*Human Gene Arrays 1.0*, Affymetrix), by sigmoidal dose-response curves. Western blotting was performed with a monoclonal mouse anti-human PR antibody (M3569; Dako, Hamburg, Germany) and with a polyclonal mouse estrogen sulfotransferase (SULT1E1) antibody (Sigma, Taufkirchen, Germany).

Results

In experiments with mifepristone the SULT1E1 and the PR were identified by microarray analysis as appropriate gene expression biomarkers for antiprogestin compounds. The effects on SULT1E1 mRNA were stronger than on PR mRNA. In these experiments

cells were treated with progesterone alone (10^{-8} M) and progesterone/mifepristone (10^{-8} M, each).
In our Ishikawa model for antiprogestins we observed in the initial priming phase an up-regulation of the PR by 17β-estradiol, but no effect on SULT1E1. After incubation with progesterone alone in the subsequent test phase we found a dose-dependent up-regulation of SULT1E1 mRNA and a down-regulation of PR mRNA. In combinations of progesterone (10^{-7} M) and SPRMs these progesterone effects were dose-dependently antagonized by mifepristone and ZK137316 (EC_{50} approx. 10^{-9} M, respectively). In the presence of increasing concentrations of SPRMs SULT1E1 was down-regulated and PR mRNA up-regulated. For the chemicals 4-nonylphenol and bisphenol A as well as the plant compound apigenin similar effects were observed at higher EC_{50}-values ($EC_{50} > 10^{-6}$ M). Methyl acetoacetate was used as negative control and displayed no effects. The effects of mifepristone on PR and SULT1E1 were confirmed on the protein level by Western Blotting.

Discussion

In this study we present a human endometrium-specific *in vitro* model for detection of anti-progestin effects. Compared to environmental estrogens, chemicals acting as progesterone receptor ligands have received little attention. The risk that environmental chemicals act as PR antagonists and disturb embryo implantation was hitherto not sufficiently addressed. For our study, we selected the human endometrial Ishikawa cell line. It is one of the best-characterized human endometrial cell lines currently available and expresses both functional estrogen and progesterone receptors. These properties make Ishikawa cells an ideal model to study the responses of the human endometrial epithelium to hormones and endocrine disrupters (11-12). In this study we demonstrated that our Ishikawa model is appropriate to identify antiprogestin compounds and to characterize them by sigmoidal dose-response curves. It can differentiate between strong and weak PR antagonistic effects and correctly recognize negative substances. Down-regulation of SULT1E1 and up-regulation of PR by PR antagonists in Ishikawa cells were also described by others (13-14). In summary, our Ishikawa *in vitro* model is suitable to study quantitatively effects of SPRM-like chemicals on endometrial target genes. It may be integrated into new *in vitro* test batteries developed for reproductive toxicity testing (15). Our findings demonstrate that wide-spread environmental chemicals exert weak PR antagonistic effects on the human endometrium. There is a need to screen more chemicals for antigestagenic effects (16), and for the respective risk assessment the expertise of gynaecological endocrinologists is demanded.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Effects of testosterone in human umbilical vein endothelial cells

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Introduction

Androgens are the most abundant sex steroids in men as well as in women after menopause and are also used pharmacologically. A growing body of evidence suggests that androgens affect the behavior of endothelial cells, modulating proliferation, migration and angiogenesis. The results of different studies remain contradictory with regard to the involvement of Androgen Receptor (AR) in Testosterone (T) mediated signaling. In a male human umbilical cell line EA.hy926, Ling et al (1) showed that T at concentrations from 1-100 nM, enhances apoptosis related damage under serum deprivation for 48hrs and this was abolished by the AR antagonist flutamide 0,1 μM. DHEA, an androgen which does not bind AR, has also been shown to inhibit proliferation (Mohan PF 1997, Zapata E 2005) by enhancing the expression of p53 and p21 in an AR independent manner (2). Other groups have shown that androgen stimulates proliferation of endothelial cells such as primary human aortic endothelial cells (3) and endothelial cells from human myometrium, HMMEC (4). The mechanisms implicated in the regulation of endothelial cell behavior are far from being thoroughly understood.

Hypothesis

Androgens can modulate the proliferation, migration and angiogenesis in HUVEC by an AR mediated mechanism. Raf-1 is an important effector of androgen mediated effects on HUVEC.

Materials and methods

The experiments were performed in primary human umbilical vein endothelial cells (HUVEC). The proliferation rate under increasing doses of testosterone (T) from 10^{-12} M to 10^{-6}M was studied by colorimetric BrDU ELISA. In addition we did RT-PCR, Western Blot, Boyden Chamber migration assay, hang-drop spheroid sprouting assay and ELISA for VEGFA and VEGFR2. To examine whether the changes in cell behavior were due to apoptosis, we performed an apoptosis array.

Results

Our data show that under T (10^{-6}M) the number of cells proliferating as assessed by BrDU ELISA as well as the total number of cells after 24 hours treatment, is diminished in comparison to control. Treatment with T at lower concentrations, from 10^{-7}M until 10^{-12}M did not influence the proliferation rate.
The migration rate was also lower under T treatment in comparison to 0,1% ethanol treated cells, not only at pharmacological but also at physiological levels.
We made spheroid hang-drop sprouting assay to find out whether testosterone treatment at 10^{-6} and 10^{-8}M concentrations affects sprouting as measured by total sprout length but we could not find any difference in comparison to control.
Treatment with AR antagonist flutamide (1μM) in combination with T, abolished the T-induced effects on HUVEC proliferation. T administration was associated with an increase of AR protein expression. As androgens are shown to induce the expression of

VEGF in endothelial cells (3), we performed ELISA to detect VEGFA levels. The stimulated cells do not secrete VEGFA and the total level of VEGFR2 does not change when treated with testosterone. To get insight into the mechanism by which T-treatment modifies proliferation, we performed an apoptosis array. We observed that in comparison to control, that FAS and HSP60 expression was upregulated under treatment with T 10^{-6}M. Another target that was downregulated under T 10^{-6}M treatment as assessed by apoptosis array, is XIAP which has a role in inhibiting apoptosis. On the other side, the upregulation of p21, points at cell cycle arrest.

Therefore we speculate that the upregulation of FAS leads to an increased susceptibility to apoptosis and the downregulation of proliferation is due to an arrest of the cell cycle.

In conclusion, our preliminary data confirms that the regulation of HUVEC proliferation is AR dependent. Furthermore we plan to elucidate the mechanism of T-mediated modification of endothelial cell behaviour.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Anti-Mullerian hormone (AMH): prognostic value in assisted reproductive technology (ART)

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Infertility affects about 15-20% of couples worldwide. The most common causes of infertility are: male factor such as sperm abnormalities, female factor such as ovulation dysfunction and tubal pathology, combined male and female factors and unexplained infertility (1). Best possible assessment of the ovarian reserve (OR) represents a core issue in modern infertility care in terms of prediction of ART outcome. Determination of the OR includes traditional markers such as age, FSH, estradiol (E2) and antral follicles count (AFC). Recently, AMH has been proposed as a new marker. AMH is a dimeric glycoprotein, member of the transforming growth factor-beta superfamily. Its most clearly defined role is in male sex differentiation. AMH is produced by fetal Sertoli cells at the time of testicular differentiation, and induces regression of the Mullerian ducts. In the absence of AMH, the Mullerian ducts develop into the uterus, fallopian tubes and the upper part of the vagina. In the ovaries of female fetuses, AMH expression has been observed as early as 32 weeks gestation in humans (2).

AMH is expressed by granulosa cells of early developing follicles (primary, pre-antral and small antral follicles), ending when they reach a diameter of 2-6 mm. Small antral follicles are likely the primary source because they contain larger numbers of granulosa cells. AMH has paracrine/autocrine action in follicle development but it is also measurable in the serum. The number of small antral follicles correlates with the size of the residual follicular pool. AMH levels are almost undetectable at birth, but levels increase, peaking during late puberty and then show a progressive decline throughout reproductive life, due to the depletion of the follicular reserve. In the end AMH becomes undetectable after menopause. Thus it could represent a valid estimator of OR. Because AMH derives from preantral and small antral follicles, levels are gonadotropin-independent and exhibit no variation within and between cycles. The transition from primordial into growing follicles becomes enhanced in the absence of AMH. Thus AMH levels reflect the continuous FSH-independent non-cyclic growth of small follicles in the ovary and inhibit the exhaustion of the primordial follicle pool (2,3).

The aim of this study was to confirm the role of AMH as a marker for OR and as prognostic factor of ART outcome in infertile women in terms of mature follicles, retrieved oocytes, embryo number, pregnancy rate, compared to other traditional methods. Furthermore, it was studied the correlation between AMH and total doses of exogenous gonadotropin (EG) needed to induce ovulation, and the usefulness of AMH levels in predicting poor response to ovarian stimulation.

Materials and methods

Among patients admitted to Department of ART of Spedali Civili of Brescia (Montichiari), 46 infertile couples underwent endocrine and echographic evaluation (AFC) in early follicular phase of the cycle: FSH, E2 (ARCHITECT Analyzer, Abbott Diagnostics Division), AMH (EIA Immunotech-Beckman Coulter Company, Marseille). Women were classified in normal or poor responders on the base of traditional methods: women with AFC <8 and FSH levels between 11 and 15 m UI/ml were classified as “poor responders”. “High responders” women were excluded.

Data were expressed as mean value and standard deviation. The correlation analysis has been performed with RHO-Spearman Test among endocrine, echographic and clinical parameters.

Results

The causes of infertility were: ovulation dysfunction 11 (24%), organic gynecologic pathologies (such as tubal, uterine pathology, endometriosis) 9 (19%), male factor 34 (74%), combined male and female factors 11 (24%) e idiopathic 1 (2%).
The traditional OR evaluation showed a mean FSH of 6.2±2.6 mIU/ml, E2 of 46.3±29.5 pg/ml, AFC of 5.1±3.2. Whereas AMH was 1.7±1.3 ng/ml. In particular, AMH changed between 0.4 and 3 ng/ml.
The mean women age was 35.8±4.2 years and it was between 32 and 38 years and the mean BMI was 22.4±4.2 kg/m2.
Fifty-nine percent of women underwent FIVET and 41% ICSI. Treatment was cancelled in 8 participants (17%) for different causes such as hyper-response (4%), no response (4%), drugs allergy (2%) luteal cysts (4%). Twelve (26%) single pregnancies were obtained but 2 of them (4%) miscarried. The mean number of obtained mature follicles after ovarian stimulation and the mean number of retrieved oocytes were 3. The mean number of obtained and transferred into the uterus embryos was 2.
Correlations between AMH and endocrine, echographic and clinical parameters were investigated. No significant correlation was found between age and AMH, E2, FSH, antral follicle number, mature follicle number, mature oocytes number, embryo number. This can be explained by the narrow age range. FSH showed a negative correlation with AFC (r = –0.35, p<0.02), thus the highest FSH level was associated with the lowest AFC. There was no correlation among FSH and mature follicles or retrieved oocytes.
AMH showed a significant positive association with AFC (r = +0.59, p<0.001), mature follicles at ultrasound (r = +0.44, p<0.002), retrieved mature oocytes (r = +0.39, p<0.02) and negative with FSH (r = – 0.43, p<0.003) and E2 (r = –0.31, p<0.05). Thus AMH is a marker of OR and predictor of response to ovarian stimulation. Pregnancy rate had a significant positive association only with mature follicles after treatment (r = +0.47, p<0.005), and so indirectly with AMH, and with developed embryos (r = +0.36, p<0.04) (Table 1).
The mean age and FSH of "poor responders" were higher than mean value of "normal responders", on the contrary AFC and AMH. There was a negative association between AMH and exogenous gonadotropin (EG) administered to obtain ovarian stimulation. The lowest AMH levels needed the highest doses of EG (r = -0.54, p< 0.001 for exogenous FSH and r = -0.49, p < 0.01 for exogenous LH). There was no significant correlation between EG used and the other markers (FSH, E2 and age). This result indicates that AMH may be a superior marker for predicting ovarian response and total doses of EG (Fig. 1).

TABLE 1.

Parameters		R	*P*
AGE	–	–	*n.s.*
FSH	Antral foll. num.	-0.35	*0.02*
AMH	FSH	-0.43	*0.003*
	E2	-0.31	*0.05*
	Antral foll. num.	+0.59	*0.001*
	Mature foll. num.	+0.44	*0.002*
	Mature oocyt. num.	+0.39	*0.02*
PREGNANCY	Mature foll. num.	+0.47	*0.005*
	Embryo num.	+0.36	*0.04*

Discussion

In conclusion, AMH is a valid marker of OR even in the infertile woman, and it has the same level of accuracy as traditional methods. The advantage of AMH is its role as a cycle-independent test, thus it is not necessary the measurement in early follicular phase. It is not influenced by administered drugs and central anovulatory dysfunctions. Assessment of the OR is relevant in ART clinic, where moreover AMH may be useful as predictor of the extremes of ovarian response to EG.
The predictive value of AMH for ovarian response concerns mature follicles and retrieved oocytes. An interesting field of application is the classification of

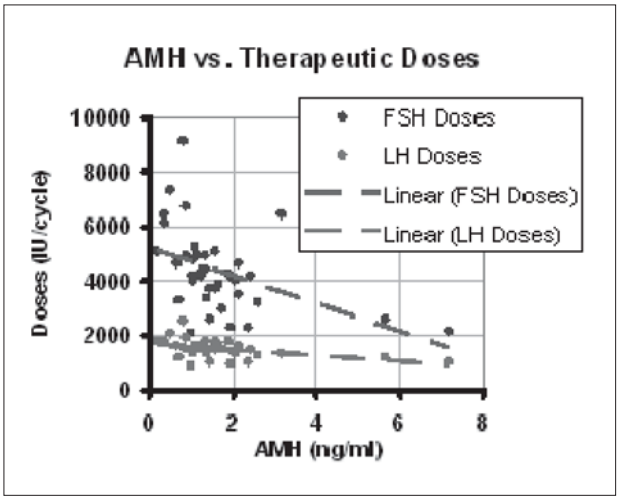

Fig. 1

women in “poor” or “normal responders” and the individualization of treatment strategy on the basis of AMH, in order to reduce the incidence of cycle cancellation and hyper-response (4).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Alternative insulin-sensitizers (Lipoic acid and Inositol) in polycystic ovary syndrome (PCOS)

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy, that affects 6-10% of women of reproductive age. It is characterized by chronic oligoanovulation, hyperandrogenism and polycystic ovaries.
Insulin resistance and hyperinsulinemia affect 50-70% of PCOS and they have a key role in the pathogenesis of the syndrome. Insulin resistance worsens clinical features of PCOS and causes metabolic morbidities (1). Insulin-sensitizers (ISs) represent a safe and effective long-term treatment in PCOS women, exerting both metabolic and endocrine beneficial effects.
ISs are involved, at different levels, in insulin's post-receptor signal transduction. Both α-lipoic acid and inositol have as a final effect an improvement in the translocation of GLUT4 on cell membrane and they ameliorate glucose utilization. Alpha-lipoic acid is a potent antioxidant and regulator of the intracellular redox status. It is able to improve insulin receptor activity and influence its pathways (1). Only one study evaluates α-lipoic acid administration in PCOS and it suggests that this drug is effective in ameliorating insulin sensitivity and lipid pattern, but no hormonal parameter was considered (2). Inositol is involved in the phosphatidylinositide pathway and it seems to be lower in women with PCOS (3). Some studies suggest that inositol administration is effective in ameliorating PCOS dysmetabolic features (insulin resistance, dyslipidemia, hypertension, weight) and endocrine profile, leading to menstrual pattern regularization and restoring fertility (3).
The aim of this study was to assess ISs' efficacy and safety in PCOS treatment, and to evaluate the effect on clinical, hormonal and metabolic parameters.

Patients and methods

Among patients admitted to the Department of Gynaecological Endocrinology of the University of Brescia/Spedali Civili, we selected 19 overweight PCOS (BMI 25-29 kg/m^2), not previously treated with OC, antiandrogens or metformin. Before treatment, they underwent clinical, endocrine-metabolic and echographic evaluation.
The patients underwent the evaluation of Body Mass Index (BMI), menstrual pattern, Ferriman-Gallwey and Rosenfield scores, serum LH, FSH, 17-β estradiol (E), androstenedione (A), total and free testosterone (T, fT), Sex Hormone Binding Protein (SHBG), deidroepiandrosterone (DHEAS), total cholesterol, HDL cholesterol (HDLc), LDL cholesterol (LDLc), triglycerides, glycemia, insulinemia, HOMA index and ovarian volume.
Patients were randomly treated for 6 months with α-lipoic acid (9 patients with 600 mg fast-slow, twice daily, Tiobec®, Laborest – Milano) and inositol plus antioxidants (10 patients, with 1500 mg once daily, Redestop®, Progine – Firenze). Clinical changes were evaluated after 3 months of therapy and endocrine-metabolic and echographic modifications after 6 months of therapy.

Results

After 6 months of therapy, α-lipoic acid reduced A (-20%), fT (-45%) and increased E (+17%) and SHBG (+30%). Furthermore, it reduced ovarian volume by 33%. Inositol determined a reduction in LH (-35%), A (-19%), total testosterone (-25%), fT

(-56%), and increased SHBG (+15%). It also reduced ovarian volume by 10% (Table 1).

TABLE 1 - MODIFICATION OF ENDOCRINE PROFILE AND ECHOGRAPHY AFTER 6 MONTHS OF TREATMENT.

	α-lipoic acid	Inositol
LH	=	-19%
FSH	=	=
E	+17%	=
A	-20%	-19%
T	=	-25%
fT	-45%*	-56%*
SHBG	+30%	+15%
Ovarian volume	-33%*	-10%

Student t-test *P<0.05 after vs before treatment.

Alpha-lipoic acid and inositol determined mild changes in metabolic parameters, anyway it should be noted that patients did not have altered mean insulin-sensitivity or lipid values in basal conditions. After 6 months of therapy α-lipoic acid decreased glycaemia (-8%) and HOMA index (-15%). Inositol reduced glycaemia (-4%), insulinemia (-14%) and HOMA Index (-19%). Only two patients (one from the α-lipoic acid group and the other one from the inositol group) had pathologic values of HOMA Index before treatment and they both had a regularization after six months of therapy. Furthermore, three patients were dyslipidemic in basal conditions and two of them, treated with inositol, ameliorated their lipid pattern after 6 months of therapy.

Clinical results show that ISs are effective in allow weight loss after a 3 month treatment. In particular, 67% of patients treated with α-lipoic acid and 50% of those treated with inositol lost weight. The mean weight loss was -3.3 kg (range from -1 to -7 kg) for the first group and -2.0 kg (range from -1 to -4 kg) for the second.

ISs determined regularization of menstrual pattern after 3 months of therapy: 56% of patients treated with α-lipoic acid and 40% of those treated with inositol showed regular ovulatory menstrual cycles. Short-term therapy with ISs showed to be able to treat mild hyperandrogenism clinical features such as acne.

Side effects affected one patient treated with α-lipoic acid and one treated with inositol. In both cases, patients complained about mild nausea and diarrhea.

Discussion

In this study alternative ISs demonstrated to be effective in modifying endocrine-metabolic profile of overweight women affected by PCOS, determining menstrual pattern regularization and weight loss. Furthermore, α-lipoic acid and inositol were safe and did not show severe side effect, thus they are easily manageable and effective therapy for PCOS.

Alternative ISs (α-lipoic acid and inositol) might be considered as the first-choice long-term therapy for mild to moderate PCOS. In particular, they should be used in non-obese patients, without severe clinical signs of hyperandrogenism or insulin-resistance and no need of contraception. They are safe and effective both on endocrine and metabolic alterations of PCOS, and could prevent long-term morbidity linked to the syndrome.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

In vitro evaluation of the protective and reparative effect in relation to damage caused by external agents on the vaginal epithelium

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Introduction

The female genital tract is particularly exposed to many possible types of attack due to the close contact with the external environment. In order to combat the proliferation of pathogens and micro-organisms and in general to activate defences, there is a natural "barrier" comprising the external surfaces (vaginal epithelium and mucus) and the vaginal ecosystem, which maintains an acid milieu. Changes in this system may be due to physiological, behavioural or environmental causes.
Whenever induced stresses exceed the natural defence capabilities of the female genital tract, infections and inflammation may develop.
Good feminine hygiene is essential to prevent irritation of the vulva and vagina.
Therefore, identifying and validating substances able to prevent or repair damage to the vaginal epithelium is of clinical and scientific interest.
The objective of this study is to establish an in vitro method for efficient, straight-forward and rapid evaluation of substances and systems potentially able to protect the vaginal epithelium from damage due to external agents and/or to repair such damage.
In order to do this, an organotypic culture of vaginal epithelium was used to monitor changes in some biomarkers associated with damage caused by external stimuli (surfactants) such as the change in cell vitality and evaluation of the release of pro-inflammatory cytokines in the culture medium (IL-1 alpha).
Analysis of the experimental data showed that this model is able to evaluate and differentiate substances both in terms of their reparative potency in the case of studies on products to be applied following damage and the protective effect in the case of products applied preventively and, lastly, the effect of repeated application of these products.

Materials and methods

Organotypic cultures of vaginal epithelium (Vec-100 EpiVaginal™ MatTek) were stimulated using a non-inonic surfactant (1% Triton x100 MatTek) to simulate damage caused by external agents and treated (preventively, post-injury and on a repeated basis) with two substances. The tests used are the MTT test from Sigma-Aldrich (Gerlier & Thomasset, 1986) to monitor cell viability and an ELISA test (Bender Med Systems®) to assay IL-1 alpha.
In order to evaluate the sensitivity of the method, two natural polysaccharide substances were used which, for simplicity's sake, we will call substance A and substance B.
All experiments were repeated at least 3 times, with representative results shown. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using InStat software version 3.0a (GraphPad Software, La Jolla, CA, USA). Statistical differences were determined using ANOVA followed by a multiple comparison test. Effects were designated significant if $p<0.05$.

Results

Tissue compatibility (Fig. 1)
This first analysis was undertaken to verify that the

two substances (A and B) have no intrinsic toxic effect and to check whether the dose of Triton X100 (1%) is sufficient to reveal damage. The two substances selected were applied on EpiVaginal for 18 hours, and a 12-minute insult with Triton X100 was used as a positive control while the untreated tissue was used as a negative control. The results reveal the toxic effect of Triton X100 and the absolute compatibility of the substances with EpiVaginal.

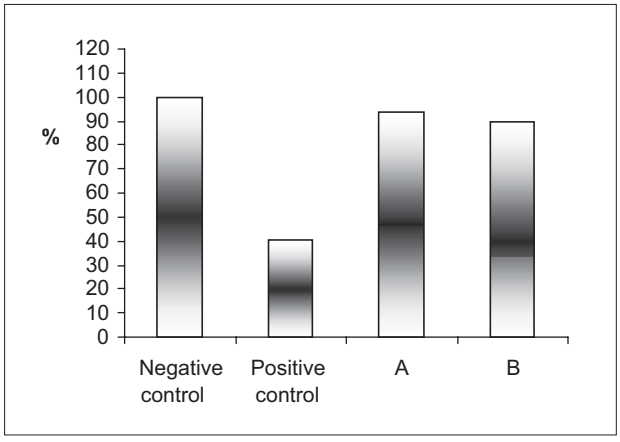

Fig. 1

Treatments: preventive (Fig. 2), reparative (Fig. 3) and repeated (Fig. 4)

The treatments were:

– preventive treatment (Fig. 2) involving 1 hour of pre-treatment followed by insult with Triton X100 for 12 minutes, rinsing, and 18 hours' incubation
– reparative treatment (Fig. 3) involving a 12-minute insult with Triton X100, rinsing, treatment with the substances for 18 hours
– repeated treatment (Fig. 4) involving 1 hour of pre-treatment followed by a 12-minute insult with Triton X100, rinsing, further treatment with the substance for 18 hours.

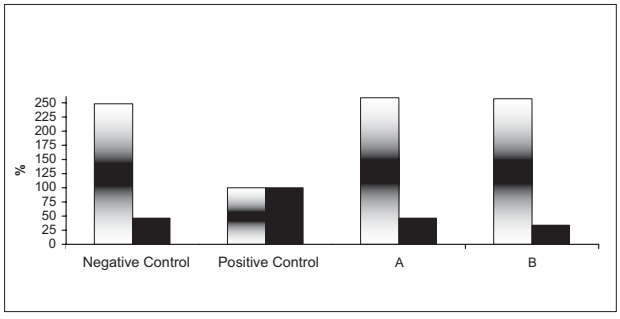

Fig. 2

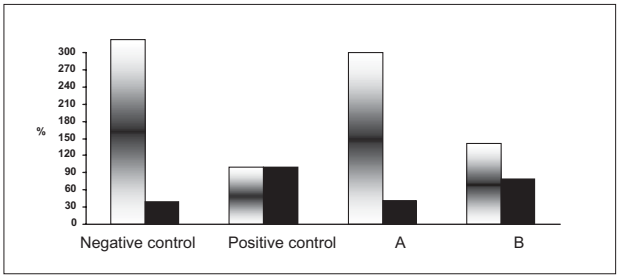

Fig. 3

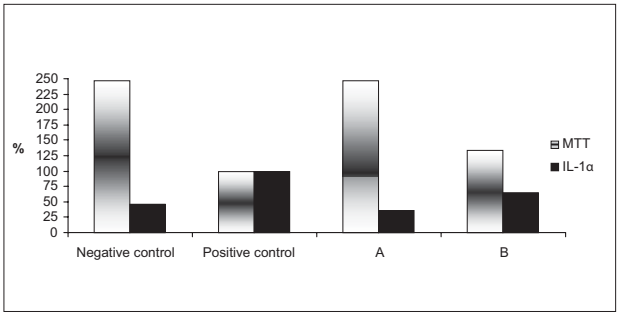

Fig. 4

As can be seen in Figure 2, the two substances show excellent protective potency, while in terms of reparative properties substance A has greater potency (Fig. 3). On repeated treatment, substance A allows optimal protection almost at untreated levels while substance B has a slight negative effect on the viability and release of IL-1α (Fig. 4).

Conclusions

Analysis of the experimental data showed that this model is able to evaluate and differentiate substances both in terms of their reparative potency in the case of studies on products to be applied following damage and the protective effect in the case of products applied preventively and, lastly, the effect of repeated application of these products.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Seminal plasma proteomics for the identification of novel fertility markers

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Introduction

Human seminal plasma contains many proteins that are important in the capacitation of the spermatozoa, in the modulation of the immune responses in the uterus, in the formation of the tubal sperm reservoir and finally in both the sperm-zona pellucida interaction and in the sperm and egg fusion. The complex content of seminal plasma allows the successful fertilization of the oocyte by the spermatozoa. Some of the seminal proteins are secreted by the testis, epididymis, and male accessory glands such as seminal vescicle, prostatic ampulla, and bulbourethral glands. Apart from these organ-specific proteins, human seminal plasma is rich in other proteins, whose origin and function are not completely clear.
The aim of the present study is to analyze human seminal plasma proteome of fertile men, by LTQ-Orbitrap mass spectrometer, in order to identify a panel of common seminal proteins in fertile men.

Materials and methods

Five fertile men, whose partners were pregnant when the study was started, participated in this study. In all patients, a standard semen analysis was performed according to WHO classification.
Liquified semen samples were then centrifuged at 9,200x g for 20 minutes to obtain the seminal plasma. An aliquot of each seminal sample was subjected to in solution digestion protocol. The samples obtained from the digestion procedure were resuspended in aqueous trifluoroacetic acid and analyzed with an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module, coupled with an LTQ-Orbitrap XL hybrid mass spectrometer.
Tandem mass spectra were analyzed by the Thermo Proteome Discoverer 1.2 software, using SEQUEST as a search engine against uniprot-taxonomy-9606_human database. Protein informations were then analyzed using the software tool for researching annotations of proteins (STRAP), to annotate each protein according to the gene ontology (GO) system (1). When an individual protein was assigned to more than one GO annotation, all of the annotations were counted nonexclusively.

Results

All subjects had hormonal values within the normal range. Semen parameters showed normospermic condition of all subjects.
Protein identification criteria resulted in the identification of 919 to 1,487 unique proteins per individual subject sample and 83 proteins were present in all samples.
The largest proportion of GO annotations for molecular function were binding proteins, which occurred in 54 proteins (58%). Twenty proteins were annotated as involved in catalytic activity, 10 in structural molecule activity, and 1 was involved in enzyme regulation. Other activities were reported for eight proteins.
Analysis of GO cellular distribution annotations is reported in Figure 1. Biological process analysis of the proteic pattern is reported in Figure 2.

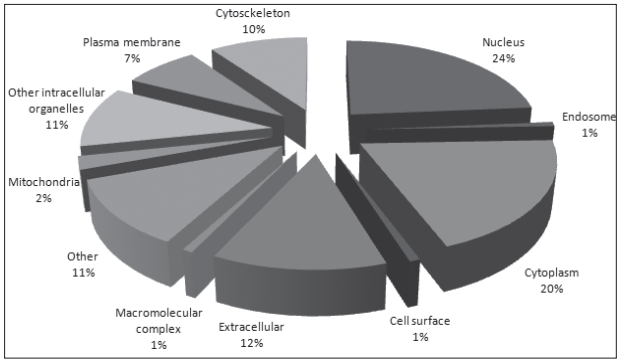

Fig. 1 - Go annotations for cellular distribution.

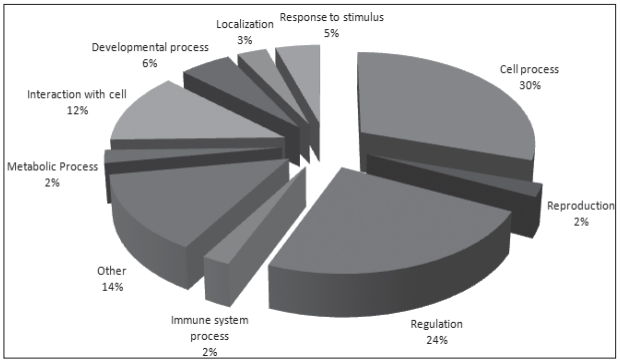

Fig. 2 - GO annotations for biological process.

Discussion

The importance of knowing the composition of seminal plasma is essential in understanding the physiology of reproduction. We identified 83 common proteins in seminal plasma in a group of fertile subjects. This is the first identification of the common pattern of seminal proteins in male fertility, using high-resolution MS. We identified up to 1,487 unique proteins per single sample, showing a greater sensitivity of the procedure.

In our study, semenogelin (Sg) I and Sg II are expressed in all samples. This was predictable as Sg is the main protein of human semen coagulum and plays an important physiological role in the suppression of sperm motility at ejaculation.

Within the group of common proteins we reported the olfactory receptor (OR) 5R1. The G-protein-coupled ORs make up a large multigene family, whose ectopic expression has been related to testis and germ cells, where several dozen human and mouse ORs were shown to be transcribed (2). The ORs appear to be expressed in late spermatids and on the tail midpiece of mature spermatozoa, implying that testicular ORs are involved in either sperm maturation, migration, or fertilization (3). These results therefore led to the hypothesis that at least some ORs are involved in mammalian sperm chemotaxis. Proteomic platform may give new insight in understanding the expression and role of ORs in germ cells and seminal plasma.

With regard to the proteins identified in all patients, we identified some proteins involved in fertility and reproduction such as lactoferrin, human cationic antimicrobial protein (hCAP18), and spindlin 1.

Lactoferrin has antibacterial, antioxidative, and an immune-modulating role in seminal plasma. It is also involved in maintaining normal sperm structure and motility and modulating the composition and quality of the semen during sperm maturation and migration through the male genital tract (4).

The hCAP18 has a key role in the innate immunity of the male reproductive system. It was present in the epithelium of human epydidimis, in the seminal plasma at high concentrations, and in association with spermatozoa. It is not unlikely that the spermatozoa transport hCAP18 with them on their way to the ovum and that the hCAP18 provides protection against microorganisms during fertilization.

Spindlin 1 protein is involved in spermatogenesis in the first wave of spermatocyte meiosis. Spindlin 1 was also localized at the tail of mouse sperm and could be essential for normal sperm motility (5).

Considering the molecular function annotation of the proteins, the most abundant group include proteins involved in protein binding. However, it may represent an auxiliary role to the main one of that protein, which is closely linked to enzymatic or transport activity. Catalytic activity was in fact annotated in 22% of proteins and 1% of proteins is classified as their regulators, implying that 23% of the seminal fertile proteomic pattern is involved in enzymatic activity.

Membrane proteins are present with a frequency of 27 annotated proteins. It may depend on whether some of these proteins are bound to the sperm surface during ejaculation, thus forming protein-coated layers (6). This is confirmed by the number of membrane proteins that are annotated both as membrane proteins and as extracellular or surface proteins.

The largest group of proteins is composed of 40 proteins involved in cellular processes, followed by 31 proteins involved in regulation, depending by the presence of both enzymes involved in the regulation, processing, or degradation of seminal fluid proteins and coagulation of semen such as in the basic cellular processes.

Finally we offered a proteomic approach to identify a proteic panel for male fertility by using the LTQ-Orbitrap mass analyzer.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

2D vs. 3D ultrasound assessment for infertility evaluation on the 3rd day of the cycle

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Introduction

Sonography at day three of the menstrual cycle is mandatory during infertility work-up. This ultrasound must include the examination of the uterus and adnexa in order to identify infertility factors that might modify either the treatement or the prognosis of the patient. It also allows planning of treatment options through the evaluation of the ovarian reserve by counting the total number of antral follicles (1). The standard technique use 2D ultrasound for evaluation of the uterus, fallopian tubes and ovaries. Although its clinical importance is well defined, 2D has several limitations, which can be surpassed by 3D ultrasound. We want with our study to evaluate in which measure 3D ultrasound, when added to standard 2D ultrasound, could improve the diagnostic.

Materials and methods

In our study we examine 44 patients with primary or secondary infertility. This study was approved by the local ethics committee and written informed consent was obtained from all participants We examined this group of patients using both 2D and 3D ultrasound in day 2, 3 or 4 of the menstrual cycle with a Voluson E8 machine, 3 D endovaginal. We scanned the uterus and the both adnexa and we recorded the anomalies of the uterus for each particular case (detected by 2D or 3D ultraosund, or both), of the ovaries or fallopian tubes. We measured the total number of antral follicles, first by 2D and than automated by 3D ultrasound using sonoAVC software. For the 2D assessment of the antral follicles we used the standard technique wich quantifies the size of a follicle by the mean diameter from two linear measurements of the follicle (6). In order to examine the coronal plane of the uterus with 3D ultrasound we used the "Z" technique developed by Abuhamaz (2).

Results

We have examined 44 patients with primary infertility - 29 cases (65.9%) and secondary infertility - 15 cases (34.1%). Regarding uterine pathology, we had four cases with uterine myoma, one case with endometrial polyp, 3 cases with adenomyosis and one case with an arcuate uterus (Table 1).

TABLE 1 - UTERINE PATHOLOGY DETECTED WITH 2D AND 3D ULTRASOUND.

Uterine pathology	Nr. of cases	%
No endometrial or myometrial abnormalities	35	79.54
Myoma	4	9,00
Endometrial polyp	1	2.20
Adenomyosis	3	6.80
Arcuate uterus	1	2.20

All of these 9 cases, except one were diagnosed first by 2D ultrasound and the diagnosed was then confirmed by 3D ultrasound. In a single case with a uterine malformation the diagnosed was missed by 2D ultrasound and it was established after obtaining the coronal plane of the uterus (Table 2).

TABLE 2 - COMPARISON BETWEEN 2D AND 3 D REGARDING DETECTION OF UTERINE ANOMALIES.

Uterine pathology	Diagnosis with 2D	Diagnosis with 3D	Diagnosis with 2D and improved by 3D
Myoma	+	+	+
Endometrial polyp	+	+	+
Adenomyosis	+	+	Not the case
Arcuate uterus	-	+	Not the case

In all cases with myoma the diagnosis was made with 2D but adding coronal plane gave more information regarding the exact topography of the tumor. In one case the myoma was intracavitary and although this aspect was suggested by 2D, the 3D was more useful in confirming this aspect. The exact position of a myoma, the impact on the ostium tubae and on the uterine cavity may be difficult to assess by conventional ultrasound. Adenomyosis is a disease that regularly affects the endometrial-myometrial junction. The diagnosis is possible to be found by ultrasound if striations of the myometrium, anechoic foci in the myometrium and asimetric uterine cavity could be visualized. In our cases the diagnosis was evidenced with both 2D and 3D sonography.

Regarding the antral follicle count we performed a double measurement. First we counted them on 2D ultrasound using the standard technique. We measured each follicle with a mean size between 2 and 10 mm. Because some authors consider that is it best to count the follicle up to 8 or 9 mm we analyzed separately these sizes (Table 3).

TABLE 3 - NUMBER OF ANTRAL FOLLICLE WITH 2D AND 3D ULTRASOUND.

Follicle size	2D mean±SD	3D mean±SD	Mean difference
2-8 mm	17.51±11.51	16.33±12.13	1.18
2-9 mm	18.24±11.51	16.67±9.68	1.57
2-10 mm	18.82±10.75	17.18±9.75	1.64

We found no statistical significant difference between the two methods (2D vs 3D) regarding the number of follicles. We must emphasize although that the 3D count is obvious easier than the 2D method.

Discussion

This study intended to evaluate in which extent adding 3D ultrasound at the day 3 ultrasound of an infertile female could bring new and valuable information. Conventional 2D ultrasound, with a thorough scan in both sagital and transverse sections, offers an almost complete description of the uterus, endometrial thickness and vascularisation pattern. Normal uterus is easily assessed using 3D ultrasound, while the coronal plane gives a good image of the endometrial cavity, the surrounding myometrium and of the uterine external contour, a fact of most importance. 3D ultrasound does not substitute, but completes the examination by offering a complete image of the uterine cavity in one single data acquisition (3).

2D ultrasound presents the endometrial polyp as a focal, unequal thickening of the endometrium, of higher echogenicity than the myometrium, with an easily detectable single feeding vessel (4). 3D sonograpahy may facilitate diagnosis, using static acquisition, in direct or inversion modes. Also, the shape, the dimensions, the origin and the impact on the endometrial cavity are clearly visualized, guiding the therapeutic procedure. In our case the polyp was diagnosed during 2D examination and confirmed by 3D sonography and the coronal examination also confirmed the diagnosis.

Uterine myomas are present on 2D ultrasound as focal enlargements of the uterus with a texture similar to the myometrium and posterior shadowing. The exact position, the impact on the ostium tubae and the uterine cavity may be difficult to assess by conventional ultrasound (5). A very easy solution in many cases is offered by a static 3D acquisition or static VCI-C. In our case with submucous myoma, the 3D ultrasound showed clearly its position inside the uterine cavity.

Although the incidence of uterine anomalies is evaluated in the literature as 7.7% and even higher in population with infertility, in our particular study we diagnosed only one case with arcuate uterus (7,8). It is important to emphasize that in our case the diagnosis was missed on 2D ultrasound and only after obtaining the coronal plane this uterine malformation was clearly seen. Uterine anomalies are undoubtedly the area where 3D sonography has contributed the most. While 2D transvaginal sonography is an excellent screening examination for uterine anomalies, it is not as effective as 3D ultrasound in distinguishing specific malformations. 3D ultrasound does not replace 2D ultrasound but, rather, enhances it.

Another important element evaluated during the day 3 ultrasound is ovarian reserve (9). It is estimated that the number of primordial follicles in the ovary is in direct correlation with the number of growing antral follicles (2). Different studies using transvaginal ultrasound have shown decreasing numbers of antral follicles as visualized by transvaginal sonography related to

increasing age in both women without known fertility problems and in women with proven natural fertility (10). The predictive value of antral follicle counts (AFC) towards the probability of pregnancy in couples with more or less unexplained infertility or in couples in whom there is an indication for assisted reproductive procedures, has been the subject of many studies. Counting the true number of follicles with 2D transvaginal ultrasound could sometimes be difficult (10). With the recent availability of three-dimensional (3D) ultrasound equipment the assessment of the antral follicle number may become more precise. Moreover, computerized counting on the basis of digitalized three-dimensional recordings is a new a possibility today. We found no statistical difference in the number of antral follicle by 2D or 3D ultrasound. The difference could be in the case of measuring automated the follicle, the method beeing far more rapide than the manually count by 2D (11).

Conclusions

Ultrasound examination of any patient performing an infertility workup is an integral part of the standard patient management. This is always done by 2D transvaginal ultrasound. It allows proper examination of pelvic organs: uterus, ovaries and possibly tubes. In the last decade 3D ultrasound has been extensively developed and applied and the field of its use has broadened. In day 3 ultrasound, the 3D sonographic examination is useful in uterine pathology, by obtaining the coronal view. It could also be used in assessing the ovarian reserve.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Evaluation of "see-and-treat" approach in women with cervical high-grade squamous intraepithelial lesions

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Introduction

Cervical cancer is a major health issue. Annually, an estimated 470.000 new cases occur worldwide, of which nearly half die. To minimize the effects of this problem, early detection and proper treatment of cervical precancerous lesions are therefore unquestionably necessary. These two critical processes, however, are still problematic in developing countries because of the limitations of national screening and treatment programs.
The "see and treat" or one-step management is an immediate treatment of cervical precancerous lesions by loop electrosurgical excision procedure (LEEP) without intervening via colposcopically-directed biopsy. This strategy provides several advantages including the simultaneous histological diagnosis and treatment of cervical precancerous lesions resulting in reduction of either the number of patient visits or of the time intervals from diagnosis to treatment, a more accurate histological diagnosis due to larger specimens than those obtained by cervical biopsy a decrease in cost and greater patient's convenience, thereby improving the compliance (1, 2, 3, 4). This study was undertaken to evaluate if "see and treat" management in women with HSIL Pap smear represent a good medical practice in our medical facility.

Materials and methods

We performed an analysis of 75 cases with a cytologic diagnosis of HSIL on the Pap smear. Exclusion criteria were any proven genital malignancy, being a candidate for hysterectomy for any indication, severe vaginal infection, pregnancy, a delivery in the 3 previous months, a bleeding disorder, or a recent cervical electrocauterization.
All the patients had an colposcopic examination and the severity of the lesion was graded using the ASCCP guidelines, by which lesions with a mildly acetowhite epithelium, no blood vessel pattern, and vague, diffuse lesion borders that follow the normal contour of the cervix are less severe than intensely acetowhite lesions with punctuation or mosaic patterns, sharply demarcated borders, and a "humped up" contour (5).
We divided these patients in two groups and we applied two different management approaches. In the first group (39 patients) we used the one step "see and treat" management of the lesion and a second group (36 patients) on which we performed biopsy followed by LEEP or conization when necessary (depending on the result of the biopsy). We then compared the results of the pathology reports in order to evaluate if the "see and treat" management is not an overtreatment in some cases. In the second group we performed 34 LEEP based on the results obtained from the pathology department.
The patients presented for follow-up visits at the outpatient clinic 2 weeks, 6 months, and 1 year after the procedure to undergo Pap smear, colposcopy, and colposcopically-directed biopsy when the colposcopic examination was not satisfactory. The first visit allowed an early evaluation of the procedure, the detection of any complications and an evaluation of the patient's satisfaction. The later visits allowed the detection of residual or recurrent lesions.

Results

The demographic characteristics of the patients are presented in Table 1.

TABLE 1 - DEMOGRAPHIC PARAMETERS.

	No. of cases	%
Age group (years old)		
≥ 20	1	1.33%
20-40	60	80.00%
41-60	13	17.33%
> 60	1	1.33%
Income status		
High	23	30.66%
Medium	47	62.66%
Low	5	6.66%
Residence		
Urban	70	93.33%
Rural	5	6.67%
Onset of sex		
< 20 years of age	49	65.33%
≥20 years of age	26	34.66%
Parity		
≥ 3	65	86.66%
> 3	10	13.33%

The pathological results are presented in table 2. The hystophatological report show CIN II, III or CIS in 67 cases (89,3%). In 8 cases (10,6%) the diagnosis was CIN I or chronic cervicitis. Among these 8 patiens, 4 belonged to group 1 and 4 to group 2, so there was no significant difference of overtreatment between the two groups.

TABLE 2 - COMPLICATIONS DURING AND AFTER THE PROCEDURE.

Complication	Group 1 (39 patients)	Group 2 (biopsy guided procedure, 36 patients)	Subgroup 2 (LEEP after biopsy, 34 patients)
Bleeding during the procedure	0	0	0
Bleeding after procedure	0	0	0
Injury to vaginal mucosa	0	0	0

The complications which appeared during or after the procedures are presented in table 3.

TABLE 3 - PATHOLOGICAL RESULTS.

Pathology	CIN III, CIS	CIN II,	CIN I	Chronic cervicitis
Group 1 (39 patients)	10	25	3	1
Group 2 (36 patients)	8	26	2	0
Subgroup 2*(34 patients)	9	22	3	0

*Subgroup 2- patients with LEEP after guided colposcopic biopsy

In group 2, in 34 cases the pathology report from the colposcopic guided biopsy indicated a high-grade lesion and this result certified the need for further LEEP or conization. From the 34 cases with conization in 2 cases the final diagnosis was CIN I.
The medium time length between the initial Pap smear and LEEP was significantly longer than between the initial Pap smear and biopsy.
The histopathologic evaluation revealed that 4 patients (10,2%) in the subgroup 1 have been overtreated, as they only had chronic cervicitis or CIN I.
The LEEP treatment was unsuccessful in 4 patients (5,1%), 2 (2,6%) in the LEEP subgroup of group 1 and 2 (5,8%) in the LEEP subgroup of group 2. Of those 4 patients, 2 had CIN 2 and the other 2 had CIN3, as evidenced on colposcopy and confirmed on histopathology.

Discussion

Tradditionally, the management algorithm of cervical precancerous lesions has involved multiple steps including initial colposcopy, followed by colposcopically-directed biopsy, and LEEP or conization thereafter depending on the biopsy result. This protocol require multiple visits and sometimes has a lower efficacy due to poor patients' compliance. In order to improve the patients' compliance, an alternative strategy using LEEP for cervical conization method without prior histogic diagnosis at the time of initial colposcopy, the so-called “see and treat” is proposed because it could reduce the number of visits compared with the traditional management. Due to its several advantages, LEEP has become the preferred conization methods among gynecological practice particularly for “see and treat” approach (6, 7).
The problem arised by the “see and treat” approach is the overtreatment of the cervical lesions. This overtreatment mainly depends on the severity of the lesions sugested by Pap smear and colposcopy. For LSIL and ASCUS abnormalities the overtreatment is highly probable. Our data strongly suggested that “see and treat” policy was an inappropriate strategy for women with low-grade cytological abnormality because of the high incidence of the overtreatment.
In our study we evaluted this approach for patients diagnosed with high-grade lesions on Pap smear. In the group where we apllied the “see and treat” approach, we had a 10,2% overtreatment rate. However, we found no statistical difference between this finding and the 11.7% rate of non-high-grade results from the LEEP specimens in group 2, after high-grade results were obtaining from punch biopsies. Based on these results we consider that the “see and treat” approach

represent a good therapeutical attitude especially when is performed in patients who cannot afford regular medical controls.

Our results agree with those of Numnum et al., who reported an overtreatment rate of 16%, with overtreatment defined as the excision of CIN 1 or lesser lesions (4). On the other hand, Ferris et al. found that the histologic results were normal for 36.1% of the patients in their see-and-treat group (2).

Sadan et al. found no differences in final histologic findings between a 3-step protocol, in which LEEP was performed, only in the cases when the results of colposcopically-directed biopsies were positive for CIN 2 and CIN 3, and a see-and-treat protocol, in which LEEP was immediately performed if CIN 2 or CIN 3 lesions were suspected on colposcopy (8).

Conclusions

In conclusion, the "see and treat" policy using LEEP can be considered as appropriate management in women with HSIL on Pap smear with an overtreatment rate of 5.8%. The incidence of overtreatment could be further reduced to 4.0% when such policy was strictly carried out only for premenopausal patients.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The combined resection of endometrium as hormonal alternative therapy

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The hyperplastic processes of endometrium take the central place among the gynaecological morbidity. Endometrial hyperplasia is one of the most frequent diseases of the uterine mucous membrane. The prevalence degree of this pathology among reproductive age of women is more than 50%. It is the cause of abnormal uterine bleeding, infertility and endometrial cancer. Nowadays hormonal therapy is the most extended method of treatment of endometrial hyperplasia. The deterioration of the population's health index significantly reduces the possibility for prescription of a hormonal treatment. It needs the search for alternative effective treatment methods for endometrial hyperplasia of women with contraindications to hormonal treatment.

The aim of our study was to compare the efficiency of combined resection of endometrium and hormonal therapy for women with nonatypic endometrial hyperplasia.We recruited 64 women aged 45-60 with endometrial hyperplasia without atypia.

Table 1 shows that the degree of extragenital morbidities among women with endometrial hyperplasia, i.e. 89,1 per cent that explains a great number of contraindications among women of this group.

TABLE 1 - EXTRAGENITAL MORBIDITY OF PATIENTS.

Extragenital Diseases	n.	%
Arterial hypertensia	7	10,93
Ischemic heart trouble	3	4,68
Varicose disease of leg	5	7,81
Chronic cholecystitis	7	10,93
Gastroenteric diseases	17	26,56
Chronic hepatitis	1	1,56
Thyroid gland diseases	6	9,37
Diseases of kidneys and bladder	4	6,25
Respiratory diseases	4	6,25
Adiposity (III – IV stages)	7	10,93
Diabetes	2	3,12

The surveyed patients were divided into two groups. The first group, which consisted of 31 women, received the hormonal treatment during 6 months. Simple nonatypic endometrial hyperplasia was diagnosed histologically to 11 patients (35.5%) and complex nonatypic endometrial hyperplasia to 20 women (64.5%) (Fig. 1).

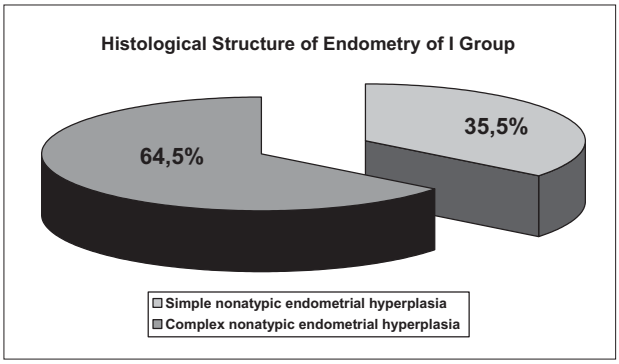

Fig. 1

The effectiveness of this hormonal treatment is 67.8%. The relapse of this disease was among 4 women during the first three months (12.9%) and among 6 women during next three months (19.3%). Thus, the recurrence of this pathology was to 10 women and that kind of treatment wasn't effective in 32.3% of the cases. The important thing is the high level of negative side effects among women who took hormonal treatment.

The combined resection endometrium was performed to 33 patients of the second group who had contraindications to using hormonal treatment or refused to take above-mentioned kind of treatment and hysterectomy.

Simple nonatypic endometrial hyperplasia was diagnosed histologically to 9 patients (27.3%) and complex nonatypic endometrial hyperplasia to 24 women (72.7%) (Fig. 2).

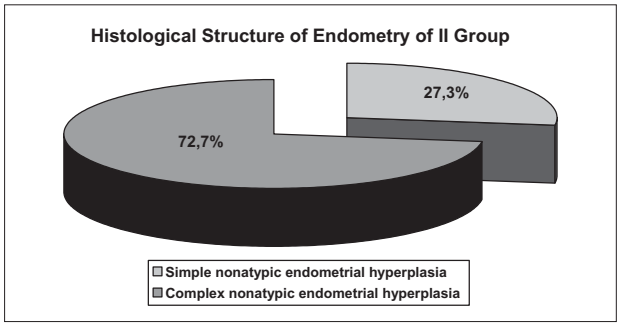

Fig. 2

The key principle of the combined resection endometrium method is the usage of different types of hysteroscopy electrodes depending on the treated part of the mucosal membrane of uterine cavity (Figs 3-5). The effectiveness of the combined resection endometrium is 87.9%. The relapse of this disease was among 1 women during the first three months (3.0%) and among 3 women during next three months (9.1%). We'd like to mark that the single side effect of the used method was a moderate pain to 7 patients (21.2%) during the first day after the operation which was easily stopped by nonsteroidal anti-inflammatory preparations. All the patients were satisfied with the results of the used treatment. There were no vegetovascular and psychoemotional disorders and flare of somatic chronic deseases that is especially important for this group of patients.

As a conclusion we'd like to say that the high degree of discomfort and side effects were determined in the progestin-treated group and according to the results of our research the sufficiency of hormonal therapy of nonatypic endometrial hyperplasia is less than 70% in comparison to 87.9% in the second group of women. On the basis of our study we believe that hysteroscopic combined endometrial resection is a safe and effective alternative to hormonal therapy for women with nonatypic endometrial hyperplasia especially for patients with contraindications to hormonal drugs and it gives the possibility to low the level of hysterectomy.

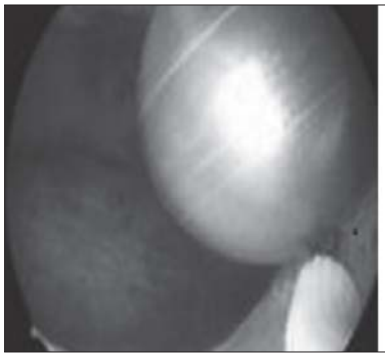

Fig. 3 - Bullet-shaped electrode, the part of internal fauces.

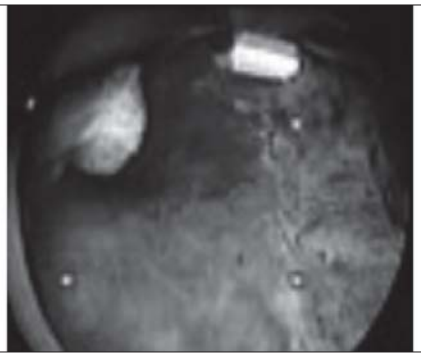

Fig. 4 - Barrel-shaped electrode, coagulation of the uterine bottom.

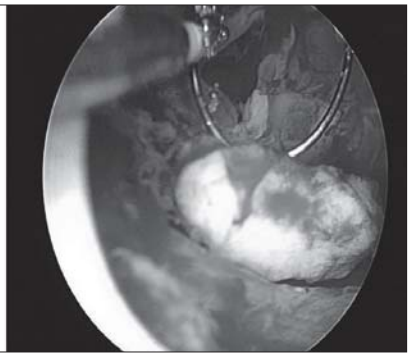

Fig. 5 - Loop-shaped electrode, posterior uterine wall.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Effects of cranial and craniospinal irradiation on reproductive system of women childhood cancer survivors

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According to recent statistics, childhood cancer incidence increases rapidly all over the world. In 2007 the cases among children in Russian Federation made upabout 11,7 o/oooo, and in 2009 about 12,9 o/oooo. Due to new prognosis of complex treatment, mortality of cancer among children is expected to decrease. The most common oncology diseases are hematological tumors and those of central nervous system. These tumors need complex treatment including surgery, chemotherapy and irradiation. However, the mentioned treatment may cause late side effects, the most common of which affect endocrine system. The relative risk of sexual dysfunction development after X-ray treatment exceeds 86 times (Gurney JG, 2003).

There are many factors that may affect reproductive function of a woman during treatment of cancer. First of all, cranial irradiation may cause complex endocrine dysfunction due to hypopituitarism. Development of secondary hypogonadism depends on dose and age and occurs at radiation dose that exceeds 40Gy.

Secondly, most chemotherapy agents are subject of high toxicity for the ovaries. Furthermore, the use of multi-agent protocols increases the risk of ovary function depletion. Chemotherapy-induced amenorrhea may be transitory, and menstruation may return several months after the treatment was completed,or it may remain permanent with total depletion of follicles.

Thirdly, ovaries are highly sensitive to irradiation. The risk of ovarian failure is high, with the LD50 estimated to be less than 2 Gy (Wallace et al., 2003). There is no direct irradiation of the ovaries during craniospinal radiotherapy (Bath et al., 2001). But ovaries may be irradiated during spinal X-ray treatment due to different types of topography. In fact, girls inpost pubertal period are more sensitive to irradiation. However, it is necessary to consider that volume of pelvis in younger girls is less than in elder ones, therefore the irradiation dose is higher.

Moreover, irradiation of the pelvis affects uterine growth as well. This may cause problems of implantation, preterm delivery and low birth weight.

Thus, different organs can be possibly impaired during the treatment of cancer.

Our study is aimed at determining the influence of cranial and craniospinal irradiation on women's reproductive system by investigating menstrual and reproductive anamnesis, ovarian volume and antral follicles count along with the level of pituitary hormones in women after treatment brain tumors and leukemia in childhood.

Methods

Medical history, physical examination, hormonal analyses. All participants were examined during the early follicular phase of a menstrual cycle (i.e. 2^{nd}-5^{th}cycle days.) with transabdominal and transvaginal ultrasound, antral follicles count and hormonal analysis (LH, FSH, prolactin, estrogen). All examinations were performed by the same investigator using the same equipment. Hormonal data, ovarian and uterus volume were compared using the Mann-Whitney U-test.

Hormonal analysis was measured in a single assay (immunochemiluminiscent system, Immulite, DPC).

The study involved 19 women.

First group included 10 women submitted to childhood acute lymphoblastic leukemia (ALL) treatment. The average age of participators in this group was

22,5(16-30), whose average age was 7,8 (2,5-13) when the diagnosis was first stated. All these patients received cranial irradiation 18 Gy and chemotherapy ALL-BFM-90 (including alkylating agents: daunorubicin, vincristine, L-asparaginase, prednisolone, mercaptopurine, cytarabine, cyclophosphamide, methotrexate, mercaptopurine).

The second group involved 9 women submitted to treatment for brain tumors in childhood. The average age of participators in this group was 19,7 (16-26), their average age by the moment of stating the diagnosis was 13,4 (5-18). This group of the patients received craniospinal irradiation 55Gy and chemotherapy M-2000 (vincristine, cyclophosphamide, cisplatin, etoposide).

None of participators received oral contraception, 2 had primary amenorrhea, 3 had secondary amenorrhea, 1 received estrogen-gestogen treatment of secondary amenorrhea. None of participants obtained gonadoprotection during treatment.

Results

Clinical data in relation to menstrual cycle characteristics showed that participants of the 1st group had menarche at the age of 11,4 years old (10-14), 5 women had clinically detected pregnancies in anamnesis. Participants of the 2nd group had menarche at the age of 12,6 years old (11-14), excepting 2 women with primary amenorrhea.

The average volume of right ovary in the 1st group was 5,56 (0,2 to 14) ml. The average volume of left ovary was 4,46 (0,2 to 9,2) ml. 8 of the patients had more than 10 follicles in each ovary during ultrasonography of ovaries. The average volume of uterus was 40,5 ml. The average level of FSH was 6,064 (5,2 to 12,4) mU/l, of LH was 4,3 (5,2 to 12,4) mU/l, of estradiol was 245,8 (73,4-1322). There were 6 documented pregnancies: 4 life-births, 1 spontaneous abortion, 1 medical abortion. All deliveries were at gestational age 37-38 weeks, average birth weight was 3462,5 (3350-3600) kg.

The average volume of right/left ovary in the 2nd group was 3,23 (0,22 to 8,76) / 4,58 (0,22 to 14,7) ml. 4 patients submitted to treatment of brain tumors had no follicles, and 4 less than 6 follicles per ovary. The average volume of uterus was 10,8 ml. The average level of FSH was 34,4 (0,19 to 105), LH was 7,1 (0,1 to 30) mU/l, average level of estradiol was 266,6 (73,0-430). There were no registered pregnancies in this group.

Discussion

This data showed that treatment of cancer in children is associated with considerable risk of disturbances of reproductive development. Women who underwent craniospinal irradiation showed elevated levels of FSH and LH. Ovarian volume was significantly smaller in women who underwent craniospinal irradiation than in those who under went cranial irradiation (3,23 versus 5,56 ml; $p<0,05$). Though the volume of ovaries did not differ significantly in this groups, there sult of antral follicles count was lower in the group of women after craniospinal irradiation than after cranial irradiation (2,3 versus 9,4 $p<0,01$). 55,5% of women after craniospinal irradiation had amenorrhea. Moreover, we have found that survivors who preserved menstrual cycles had sonographic and endocrine changes suggesting impairment of ovarian reserve.

Volume of uterus was also significantly lower after craniosponal irradiation (10,8 versus 40,5 ml, p 0,01). This could be the consequence of either spinal irradiation or amenorrhea. There were no registered pregnancies in the group of women who under went craniospinal irradiation, but none of them had ever been interested in becoming pregnant.

Conclusion

Therefore, craniospinal irradiation has a great impact on reproductive and menstrual function system of women survived cancer in childhood. Girls who undergo complex treatment of brain tumors including craniospinal irradiation are at a higher risk of amenorrhea than after complex treatment of acute leucosis. This data suggests that women after craniospinal irradiation with spontaneous menstrual cycles have reduced ovarian reserve. Further researches including direct markers of ovarian reserve indicators (AntiMullerian hormone, Inhibin B, EFFORT-test) are employed in order to estimate the risk of premature ovarian failure in women after cranial and craniospinal irradiation.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Detection of endometrial aromatase mRNA as a possible marker to screen for endometriosis and adenomyosis

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Endometriosis (ENDs) and adenomyosis (ADNs) are common gynaecological disorders with variable physical symptoms and consequences. Furthermore, the biological profile of endometriosis is known as a condition which may carry a risk for malignant conversion in some circumstances. Although ENDs and ADNs are behaviourally different diseases, they both develop in women during their reproductive ages and regress after menopause, which suggests that these diseases are estrogen-dependent disorders. The importance of aromatase expression as a specific marker for endometriosis is still a matter of debate with equivocal conclusions. Today, the diagnosis of endometriosis and/or adenomyosis is based on physical symptoms, laparoscopic and histological verification. In general, these procedures require high initial costs. We hypothesize that the estrogen dependent fashion of the disease could be of prognostic significance in diagnosis of ENDs and ADNs. The aim of this study was to determine the clinical usefulness of examining endometrial biopsy specimens for aromatase cytochrome P450 (CYP19) as a diagnostic importance for endometriosis and adenomyosis. The tissue aromatase expression level was correlated to the type of samples and to the stage of endometriosis.

Caucasian women with regular menstrual period of ages 25-55 years (mean 43.02±6.8 SD) undergoing laparoscopic surgery (tissue biopsy, suspicious endometrioma extirpation, laparoscopically assisted vaginal hysterectomy) for pelvic pain were enrolled. We divided all patients with ENDs into two stage groups: endometriosis of low stage (ENDsL; patients with stage I+II) and advanced form of the disease (ENDsA; patients with stage III+IV). The patients with adenomyosis (ADNs) were included in a separate group (n=25). Endometrial biopsies were taken by using a sharp curette (uterine cavity) and graspers (pelvic location), and stored in PrepProtect (Miltenyi Biotec, Germany) according to the manufacturer's instruction. All biopsies were obtained in the early proliferative phase of the menstrual cycle (≤7 days).

Patients (n=55) and controls (n=46) did not differ significantly neither in age, BMI nor in reproductive parameters (parity, onset of menarche and use of oral contraceptives). The increased mRNA expression of CYP19 was detected in 10 (21.7%) cases of the healthy controls and in 36 samples (65.5%) with ENDs and ADNs. In general, the Reverse Transcription-PCR analyses of 101 samples revealed increased aromatase mRNA expression in eutopic endometrium in women with ENDs and ADNs compared to healthy controls (p=0.0002). We detected the highest number of positive cases (n=14, 93.3%) of aromatase expression in patients with ENDsA. The mean value of relative CYP19 mRNA level in controls was 0.68 (interval 0.02-2.63) compared to patients with ADNs (1.21; 0.10-4.07), ENDsL stage I-II (1.15; 0.13-3.15) and ENDsA stage III-IV (1.65; 0.70-3.52), respectively (Figure 1). These results point to the highest (2.45-fold) difference in aromatase expression in patients with endometriosis stage III-IV compared to controls (p<0.0001).

Because it is thought that estrogen expression is affected by extragonadal aromatase activity, mainly in fatty tissue, we further analyzed the influence of BMI on aromatase expression in each group to achieve clearer results. Although the data showed no impact of BMI on the CYP19 level, specific and interesting trends were revealed. Despite the insignificant conclusion, the increased BMI in the controls was associated with a slight increase of aromatase expression (r=0.282,

p=0.06), contrary to cases where this trend was the opposite (r=-0.169, p=0.224).

In our study, we analyzed the mRNA expression of CYP19 in eutopic endometrium among the healthy women and patients with endometriosis or adenomyosis. A significant increased level of CYP19 mRNA in patients was revealed and this expression was significantly dependent on disease severity. These findings provide direct evidence that screening for endometrial aromatase in combination with clinical data could be of discriminative value in the prediction of estrogen-dependent diseases. Furthermore, successful treatment of unusually aggressive cases of endometriosis with aromatase inhibitors in some previous clinical trials confirmed the clinical significance of our study, bringing results that may serve also as a diagnostic marker for therapeutic intervention in women suffering from endometriosis.

Acknowledgments

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Is the ovulatory phenotype of polycystic ovary syndrome associated with adrenal hyperandrogenism?

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Introduction

The Rotterdam criteria of polycystic ovary syndrome (PCOS) include patients with different phenotypes. Ovulatory PCOS represents the mild form of classic PCOS (Guastella et al., 2010). According to Chang et al., 2005, different clinical phenotypes of PCOS may be the result of varying degree of metabolic dysfunction and greater degrees of β-cell function and circulating insulin levels favored the development of hirsutism and frank hyperandrogenemia. The main source of androgen in women with PCOS is the ovary producing mainly testosterone. However 36-50% of them have elevated adrenal androgen, dehydroepiandrosterone sulfate (DHEAS) (Carmina and Lobo, 2007). DHEAS, a weak androgen, under physiologic conditions may be converted to testosterone and then to estrogen.

Objective

To study the clinical and laboratorial findings of women with different etiologies of hyperandrogenism (HA) and to correlate with the diagnosis in the Brazilian population of São Paulo.

Patients and methods

A total of 410 patients with clinical hyperandrogenism were evaluated in the Hyperandrogenism Unit of Hospital das Clinicas of Universidade de Sao Paulo, Brazil. All patients underwent to baseline measurements of prolactin, TSH, free T4, FSH, LH, T, androstenedione, DHEAS, blood glucose and insulin and HOMA-IR calculation, and test of synthetic ACTH. Patients were classified into four groups: Anovulatory PCOS, ovulatory PCOS, Late Onset Congenital Adrenal Hyperplasia (CAH) and Idiopathic Hirsutism (IH). Rotterdam 2003 criteria were used for diagnosing PCOS; IH was diagnosed with the presence of hirsutism with all other clinical and laboratory parameters unchanged. CAH was determined by the values after stimulation with synthetic ACTH above 10 ng/mL and genotyping. Comparison of the laboratorial values among groups was performed using statistical tests ANOVA and Bonferroni.

Results

Seventy-nine percent (79%) were PCOS. From those, 13 (3.2%) were ovulatory PCOS. Thirty-two patients (7.8%) had IH and 10 (2.4%), CAH. The remaining subjects had others causes of HA (table 1). Comparing the different groups, it was observed higher age in the IH groups ($p=0.037$), higher BMI in anovulatory PCOS ($p=0.045$), and significantly higher levels of DHEAS on the ovulatory PCOS group ($p<0.001$). Abdominal circumference and level of LH, T, andro and HOMA-IR were significantly lower in IH group (Table 2).

Conclusions

Ovulatory PCOS seems to be associated with increased DHEAS, showing the influence of the adrenal component. Lower androgenic effect may explain the milder features of this PCOS phenotype.

TABLE 1 - DIAGNOSIS IN 410 HYPERANDROGENIC WOMEN.

Diagnosis	n	%
Anovulatory PCOS	312	76,1
Ovulatory PCOS	13	3,2
Idiopathic Hirsutism	32	7,8
Hypotireoidism	16	3,9
CAH non-classic	10	2,43
Hyperprolactinemia	10	2,43
Hyperthecosis	8	1,95
Androgen Producing Tumors		
Ovary	6	1,46
Adrenal	1	0,24
CAH classic	1	0,24
Cushing Syndrome	1	0,24

TABLE 2 - CLINICAL AND LABORATORIAL FEATURES BY DIFFERENT ETIOLOGIES OF HYPERDROGENISM.

Parameters	PCOS (n=312)	Ovulatory PCOS (n=13)	Idiopathic hirsutism (n=32)	CAH non-classic (n=10)	p
Age (yrs)	25,4±5,5	26,5±5,4	**28,5±9,1**	27,2±7,1	**0,037**
Menarche (yrs)	12,6±2,0	12,3±1,4	12,7±1,6	13,6±1,6	0,395
BMI (kg/m^2)	**29,1±7,3**	26,6±4,8	25,5±6,5	28,2±6,1	**0,045**
AC (cm)	90,2±15,3	84,5±11,9	**78,4±14,7**	92,0±17,4	**0,018**
Hirsutism Score (mF-G)	13,2±5,9	14,8±4,5	15,3±4,5	13,9±4,3	0,191
FSH (mUI/mL)	4,98±1,68	4,53±2,00	4,44±2,90	5,86±1,41	0,407
LH (mUI/mL)	9,84±6,29	6,95±5,72	**3,56±1,55**	9,14±7,37	**0,005**
T (ng/dL)	94,1±42,3	75,5±42,1	**49,4±16,9**	106,6±51,2	**<0,001**
Free T (pmol/L)	66,9±39,8	35,2±17,5	NA	NA	0,128
Andro (ng/mL)	3,38±1,54	3,58±2,11	**1,61±0,55**	3,45±1,44	**<0,001**
SDHEA (ng/mL)	2045±1279	**3254±1281**	1558±888	2833±1568	**<0,001**
17OHP (ng/mL)	1,44±0,95	1,44±1,40	1,87±1,05	**6,31±6,23**	**<0,001**
Glucose (mg/dL)	89,8±20,0	88,7±8,8	81,3±4,6	92,5±6,4	0,895
Insulin (µU/mL)	18,1±14,7	11,4±5,9	6,33±1,02	16,5±0,99	0,365
HOMA-IR	4,35±4,00	2,25±1,62	**1,68±0,42**	2,84±0,92	**0,049**

NA – Non available.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Does vascular endothelial growth factor improve ovarian tissue recovery after cryopreservation?

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Premature ovarian failure (POF) occurs after chemo and radiotherapy. Ovarian cortex cryopreservation followed by autotransplantation is one of the clinical options to preserve and restore fertility. Fifteen babies have been born worldwide following this procedure, however this technique still need to be optimised, especially in the ischemic early stage of the graft, source of major follicles loss. To reduce this hypoxic period, two VEGF-A isoforms ($VEGF_{165}$ and $_{111}$) were tested on ovarian graft neovascularisation.
Sheep cryopreserved ovarian cortex fragments were embedded in type 1 collagen containing or not $VEGF_{165}$ or $VEGF_{111}$. Those cortex fragments were subsequently grafted in mice.
After 3 days of transplantation, functional vascular network revealed that the percentage of perfused transplants (33%-75%) and density of functional blood vessels varied according to VEGF isoform and concentration. Nevertheless,we observed that 100% of ovarian grafts in $VEGF_{165}$ 50nM treated group were perfused and associated with a moderate increase of functional capillaries density compared to other experimental groups.
Ki-67 positive cells were localised at the ovarian graft periphery in all groups. A higher number of proliferating cells were observed in $VEGF_{165}$ group. After three weeks of transplantation, functional blood vessels and follicles were similar in all groups. However fibrosis seems to be reduced in VEGF treated groups compared to control group.
Our preliminary results show that $VEGF_{165}$ (50 nM) seems to improve early angiogenesis of ovarian grafts but requirefurther investigations.

** equally contributed*

Support

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The prevalence of endometrial hyperplasia and endometrial cancer in Danish women with PCOS or hyperandrogenism

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Objective – PCOS may be associated with an increased risk of endometrial hyperplasia (EH) and endometrial cancer (EC), but substantial evidence remains to be established. We investigated the prevalence of EH and EC in a well-characterized group of women with PCOS and/or clinical/biochemical hyperandrogenism.

Design – Retrospective, trans-sectional study.

Setting – Out-patient clinic at the Departments of Endocrinology and Gynecology, Odense University Hospital, Denmark.

Population – Nine hundred and sixty three premenopausal women consecutively referred with the diagnoses PCOS and/or hirsutism during 1997-2008.

Methods – All women underwent a standardized evaluation program. In 2011, The Danish Data Bank of Pathology was used to identify patients with endometrial histology diagnoses (year range of diagnosis 1982-2011).

Main outcome measures – Histology diagnoses, demographic variables.

Results – EH was diagnosed in 10 (1.0%) women and EC in 1 (0.1%) woman. The median BMI in the EH/EC group was 30.6 kg/m^2 compared to 26.8 kg/m^2 in the total cohort. There were no differences between the cases and total cohort in terms of individual Rotterdam Criteria. In Denmark, 70 yearly cases of EC are diagnosed in women 40-55 years corresponding to a prevalence of 0.4 % in the corresponding time period.

Conclusion – The results of the present study do not suggest a higher prevalence of EC in women with PCOS and/or clinical/biochemical hyperandrogenism compared to the general population.

Conflict of interest

None of the authors have any conflicts of interest.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

New approaches in the pathogenic mechanisms of endometrial and/or endocervical hyperplastic processes

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Background and aims

Obesity is an independent risk factor for the endometrial hyperplasia (EH) caused by a number of pathogenic mechanisms (1), including insulin resistance (IR). Hyperinsulinemia a consequence of IR is proposed to increase insulin / insulin-like growth factor (IGF) signaling in peripherial tissues, such as endometrium (2). IGF system is one of the growth factor systems. It is considered as a mediator of steroid hormone actions in the endometrium through autocrine/paracrine mechanisms (3). IR may be associated with alterations in expression of IGFs and the IGF binding proteins (IGFBPs) or may inhibit the protective effect of progestagens (3,4,5). The primary negative regulator of IGFBP-1 production is insulin (3). Insulin and IGF-1 may directly stimulate proliferation of the endometrium without the involvement of estrogen (1). The aim of this study is to compare the effectiveness of two treatment therapy - gestagen and metformin, in patients with EH and obesity.

Study design and methods

168 overweight and obese women (overweight – 29.8%, obesity of I degree – 33.9%, obesity of II degree – 36.3%) of reproductive age (from 25 to 45 years) have been studied. Patients were divided by 2 groups according to the hyperplastic processes:

- **group 1** - women with EH and/or polyposis (n=84);
- **group 2** - women with endocervical hyperplasia and/or polyposis (n=84);

Both groups were divided by 2 subgroups:

- **subgroup a** - patients on gestagen therapy from day 16 to day 25 of menstrual cycle, mean daily dosage - 20 mg, duration - 6 months (n=42);
- **subgroup b** - patients on metformin therapy, mean daily dosage 500-1500mg, duration - 6 months (n=42).

All subjects were taken a low caloric diet and performed moderate physical activity.

Inclusion criteria were: fasting hyperglycemia (FH) and/or impaired glucose tolerance (IGT); HbA1c level varied in the range 5,5-6,4%; dyslipidemia; family history of Diabetes Mellitus; birth weight more than 3.9 kg. All subjects had three and more criteria and all of them did not use other pills.

All Patients underwent to medical exams: BMI, waist circumference (WC); OGTT, HBA_{1c}, serum C-peptide, HOMA indices, serum IGF-1; FSH, LH, Estradiol, PRL, TSH; Blood coagulation test, lipidogramm; transvaginal ultrasonography (T-US); colposcopy; Pap smear test (PST); Hystomorfology. Investigations were done at the initiation of the treatment and after 6 months of the treatment termination.

Indication of diagnostic scarping and hysteroscopy was the suspicion on endometrial hyperplasia and/or polyp observed by ultrasonography and/or colposcopy. Hystomorfology revealed polypoid types of glandular-cystic hyperplasia of endometrium without atypic cells (82.7%), glandular polyp of endometrium without atypic cells (14.9%), local glandular hyperplasia without atypic cells (2.4%).

Variables were compared by the unpaired *t* test and Fisher exact test. Within-group changes were assessed by the paired *t* test. SPSS version 16.0 statistical software was used for analysis. A $p<0.05$ was required for statistical significance.

Results

After 6 month of the treatment BMI decreased non-significantly in subgroups 1a and 2a, WC – did not change.

The figures of carbohydrate and lipid metabolism were changed for the worse. At the initiation of treatment FH were noted in 32.1% of patients; IGT - in 41.7% of patients; both (FH and IGT) – 26.2%. After 6 months of treatment termination FH were noted in 26.2% of patients; IGT - in 35.7% of patients; both (FH and IGT) – 38.1%.

HOMA-IR and serum level C-peptide increased significantly ($p<0.05$); lipidogramm and blood coagulation test were worsening. Hormonal tests revealed that prolactin and IGF-1 were non-significantly slightly increased. T-US showed the decrease of endometrium thickness in 47.6% of patients and relapse of hyperplasia revealed in 52.4% of patients after 6 months of treatment termination. The incidence of varicosity of pelvic veins was increased significantly ($p<0.05$). The colposcopy data showed hyperplastic processes occurred in endocervix - micropolyposis and hyperplasia, minor size polyps, subepithelial varicose; atrophic changes, erosion in squamous epithelium. PST revealed endocervical microglandular hyperplasia and reserve cell hyperplasia, excess of parabasal cells, slightly expressed atrophic changes, LG SIL of endocervical glandular epithelial cells. Blasts of endometrial cells with proliferative activity were found in endocervical smear.

In subgroups 1b and 2b after 6 month of the treatment – reduction of BMI and WC were significant ($p<0.05$), FH have been remained in only 4.8% of patients, and IGT – 2.4% of patients.

HBA_{1c} level, C-peptide and HOMA-IR indices were decreased significantly ($p<0.05$). The results of coagulation test and lipidogram were improved. IGF-1 decreased significantly ($p<0.05$), the values of other hormones did not change significantly. T-US showed the decrease of endometrial thickness in 78.6% of patients and relapse of hyperplasia revealed in 21.4% of patients. Varicosity of pelvic veins had not been revealed. Colposcopy data showed the eradication of atrophic changes, reduction of subepithelial varicose changes, hyperplasia in cervical channel had not been revealed in 77.4% of patients. PST data indicated squamous cell metaplasia and slightly expressed reserve cell hyperplasia in 3.6% of patients.

Conclusion

Alterations in insulin/IGF signaling systems are starters of proliferation processes both for endometrial and endocervical hyperplastic processes. Insulin and IGF signaling system activation induces estrogen receptor hyperactivation. Thus, these processes are irreversible with decrease of IR. During the selection of the treatment method it is necessary to take into account as well mucosal changes as the presence of concomitant pathologies and early detection of metabolic disorders. Nowadays used hormones for the treatment of hyperplastic processes contribute to the development and worsening of the metabolic disorders and do not influence on pathogenic chain. Besides this routinely used hormone therapy has frequently short-term effect and its removal quickly leads to the relapse of disease. Metformin therapy may be a choice method for pathogenic treatment (6). It can block cell proliferation, induce apoptosis, and regulate growth factor signaling system (7). Insulin sensitizers decrease not only EH but also decline risk of cardiovascular disease and diabetes mellitus.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Evidence for the occurrence of receptor in sperm for sperm agglutinating factor isolated from *Escherichia coli*

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The significance of bacteriospermia for male subfertility has gained increasing importance in recent years. The most discussed and tested organism concerning male infertility is *Escherichia coli*, as the most important pathogen causing prostatitis and epididymitis (1). Several authors have postulated a negative effect of *E. coli* on sperm motility. The direct inhibitory effect of *E. coli* on progressive motility was found to depend upon bacterial concentration and analysis by electron microscopy revealed multiple adhesions of *E. coli* to spermatozoa causing variable ultrastructural damage as probable morphological correlates of immobilization (2).

Although several studies have evaluated the ability of bacteria to affect sperm motility by adherence, agglutination and dialyzable factors, however, none have identified receptor-ligand interaction between spermatozoa and bacteria. We have shown this same interaction using an *E. coli* strain obtained from semen of an infertile man.

The isolates of *E. coli,* showing immobilization of human spermatozoa in vitro were isolated from semen of males attending infertility clinic for semen analysis (Department of Urology, PGIMER, Chandigarh, India). The cultures were identified using Bergey's Manual of Determinative Bacteriology (3).

Semen samples were obtained by masturbation from males attending infertility clinic. Only ejaculates showing normal semen parameters according to WHO criteria (4) were used.

The screening of various isolates obtained from the ejaculates of infertile males for the interaction with human spermatozoa identified 2 isolates of *E. coli* causing agglutination of human spermatozoa. The isolate showing maximum agglutination of human spermatozoa was selected for further studies. Further, isolation of sperm agglutinating factor (SAF) from *E. coli*, was done using the method earlier standardized in the laboratory (5). Briefly, 48h old culture of *E. coli* was given salt treatment of 3M NaCl at 37°C for 14h at 150 rpm. Supernatant so obtained, was dialyzed and concentrated and applied to Sephadex G-200 column and chromatographic pattern showed that SAF was present in fractions 4-7. These fractions were pooled, concentrated and applied to DEAE cellulose column. The results indicated that most of the SAF could be eluted with PBS containing 0.4M NaCl. The bioactive fractions (31-34) when pooled, concentrated and subjected to PAGE, showed one major protein band.

Minimum effective concentration (MEC) of SAF showing complete sperm agglutination and death after 20s of incubation was determined by mixing different concentrations of SAF with human spermatozoa ($40x10^6$/ml) and results showed MEC to be 1.2 and 2.0mg/ml for agglutination and death respectively. Scanning electron microscopy (SEM) was performed to study any alteration in morphology of human spermatozoa on incubation with SAF on a Jeol scanning microscope (JSM-6100, Japan) by standard method (6). SEM studies revealed the morphological alterations of spermatozoa such as the lesions on the head region along with curling of tail after incubation with SAF.

The effect of SAF on acrosome reaction (AR) was verified using *Pisum sativum agglutinin- fluorescein isothiocyanate* (PSA-FITC) using the method of Kohn et al (7) with slight modifications. Briefly, spermatozoa were kept for 3h at 37°C in Ham's F-10 HSA medium containing Hepes (20mmol/l) for capacitation. After incubation, sample was divided in three aliquots having $1x10^6$ motile spermatozoa in each tube. Capacitated sperm were incubated with either a) DMSO

(spontaneous AR as negative control); b) CaI (physiological AR (10µmol/l) as positive control); c) SAF (0.5mg/ml; test). Further, acrosomal status was assessed by labeling fixed sperm with PSA-FITC by means of a fluorescence microscope (Nikon, Japan) and at least 200 spermatozoa were differentiated blindly according to the fluorescence pattern of their acrosomes (bright fluorescence: acrosome intact; no fluorescence or only fluorescence of the equatorial segment: loss of acrosomal membranes). The percentage of AR spermatozoa obtained in negative control (16%) after staining with PSA-FITC, were consistently lower than those incubated with SAF (88.8%) and positive control (75%).

Further, SAF was conjugated with FITC using FITC Protein Labelling Kit (Banglore Genei, Pvt. Ltd, India) to check the binding of SAF to spermatozoa and results showed florescence on spermatozoa indicating the presence of receptor on surface of spermatozoa for SAF.

Using SAF as tool, receptor on spermatozoa was isolated and purified. Briefly, washed spermatozoa were treated with 1, 2, 3 and 4M NaCl for different time intervals. The salt treated sample was then centrifuged at 1500 rpm for 20 min. Both cell debris and supernatant were dialyzed against PBS at 4°C overnight, concentrated against polyethylene glycol (PEG) 6000 and checked for blocking of agglutination induced by SAF. It was observed that the receptor of interest could be extracted by 2M NaCl when incubated for 18h at 37°C at 150rpm. The purification of receptor was carried out using gel filtration technique. Concentrated receptor was applied to Sephadex G-200 column (2 cm x 31 cm) equilibrated and eluted with PBS. Fractions (4-5) representing receptor were pooled, concentrated and analysed by SDS-PAGE. Results indicated a single protein of molecular weight ~125 kDa (Fig. 1).

Blocking studies were performed to check the reversion of adverse effects induced by SAF. The MEC of receptor required to block agglutination and death of spermatozoa induced by SAF after 20s was found to be 1.0 and 1.5 mg/ml respectively. The SEM analysis revealed no morphological alterations on human sperm when treated with ligand (1.2 mg/ml) in presence of receptor (1 mg/ml).

A comparison of the inducibility of acrosome reaction in presence of SAF showed significant differences from samples incubated with SAF and receptor. Receptor at 0.80 mg/ml could inhibit the AR induced by SAF. These results indicate that in the presence of receptor, SAF could not induce AR (Fig. 2).

Further, the enthalpy of binding of ligand with the receptor was determined in PBS using a microreaction calorimeter. Two experimental vials (reference and test) filled with 1.5ml of receptor dissolved in buffer were placed in each of the calorimetric blocks maintained at 37°C. A 250µl syringe was loaded with the buffer in case of control experiment and with ligand in case of test experiment. The experiment was conducted using the titration mode of addition of an injection of ligand solution. The binding constant, K (765/M) and enthalpy of binding, ΔH° (-10.4 kJ/mole), were computed from the experimentally calculated enthalpy of interaction between ligand and receptor, using iterative non-linear least square regression method. The values of free energy and entropy were found to be -17.11 kJ/mole and 23 J/moleK, respectively (Table 1).

In conclusion, this study identifies a receptor-ligand interaction between *E. coli* and spermatozoa that results in adverse effects on spermatozoa like sperm ag-

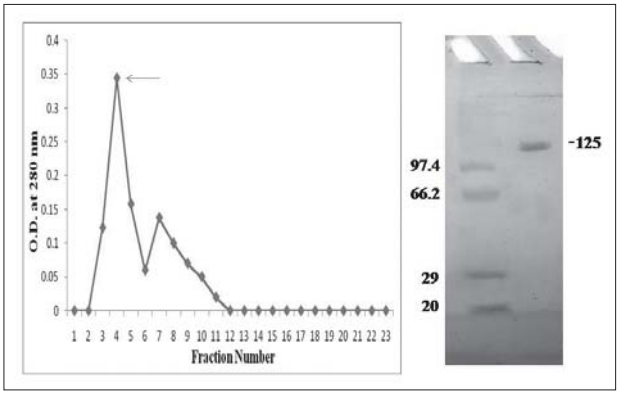

Fig. 1.

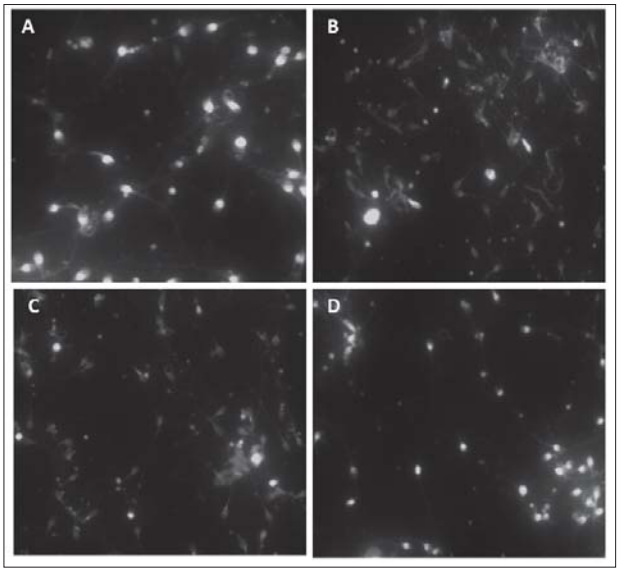

Fig. 2.

TABLE 1.

S. No.	Ligand (µmoles)	Receptor (µmoles)	Heat released (mJ)
1	$3.53x10^{-4}$	$2.4x10^{-3}$	-35.9
2	$7.04x10^{-4}$	$2.4x10^{-3}$	-99.3
3	$10.56x10^{-4}$	$2.4x10^{-3}$	-55.7
4	$2.1x10^{-4}$	$2.4x10^{-3}$	-56.3
5	$4.2x10^{-4}$	$2.4x10^{-3}$	-58.1
6	$6.3x10^{-4}$	$2.4x10^{-3}$	-57.8
7	$8.4x10^{-4}$	$2.4x10^{-3}$	-58.7
8	$11.0x10^{-4}$	$2.4x10^{-3}$	-58.6
9	$12.6x10^{-4}$	$2.4x10^{-3}$	-61.5

glutination, acrosome loss and death. The sperm agglutinating factor may be one of the important factors playing role in sperm damage during infection and isolation of corresponding receptor might act as protective measure.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Feto-placental complex marker protein concentration in patients with different genetic variants of thrombophilias

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Introduction

The success of pregnancy depends largely on adequate implantation, transformation of spiral arteries as a result of trophoblast invasion and establishing adequate blood flow in the mother-placenta-fetus. These processes are violated because of a specified hereditary thrombotic tendency when desynhronisation processes of fibrinolysis and fibrin production during implantation occur. Under the conditions that formed at the same time, the activity of proteases that are synthesized by blastocyst becomes relatively insufficient to destroy the extracellular matrix in the endometrium and immersed in sufficient depth.

Given the peculiarities of the physiological adaptation of coagulation in pregnancy, the absolute majority of genetic and acquired forms of thrombophilia is clinically manifested during the gestational process and, as it turned out, not only in the form of thrombosis, but also in the form of common obstetric complications, and the role of different forms of thrombophilia in their pathogenesis and structure varies.

Thus, according to data Makatsaryya A. et al. (9), FV Leiden mutation frequently occurs in patients with late miscarriage (15%). Prothrombin G20210A mutation occurs significantly less frequently in all groups of reproductive losses (compared with AFA, FV Leiden and MTHFR S677T) and is 4.2 and 3% in groups of early and late miscarriages.

Hormones feto-placental complex have different origins, being synthesized by cytotrofoblast and fetal adrenal glands. Changing their synthesis may indicate a violation of the processes of formation and development of the placenta and, therefore, serve as an early marker of possible development of complications of pregnancy such as IUGR, preeclampsia and premature birth. Thus, according to modern concepts, the most likely pathogenic mechanism of formation of these complications are different forms of thrombophilia and their combinations.

The significance of changes in the concentration of various hormones feto-placental complex (estriol, placental lactogen, HCG, PAPP) are different.

Often, various complications gestational process accompanied by lower concentrations of PAPP-A in maternal serum in the first trimester of pregnancy. Very difficult to answer the question of whether the content is lower serum PAPP-A compensatory response or a manifestation of this breakdown of compensatory mechanisms that ensure the safe outcome of pregnancy.

Gagnon A. and colleagues (5) published a large analytical studies conducted on materials Cochrane database and covered publications from 1966 to 2007. In the presented output shows that the inexplicable reduction of PAPP-A (<0,4 MoM) and / or β-HCG (<0,5 MoM) in the first trimester of pregnancy is associated with adverse obstetric outcomes outside depending on the therapy.

The aim of the study. To analyze the concentration of hormones feto-placental complex I and III trimesters in patients with different types of hereditary thrombophilia and their combinations.

Materials and methods

78 patients who had given a birth were examined. Patients are divided into 2 groups: The first - 61 patients, the carrier of inherited thrombophilia (factors II, V, MTHFR) and the second, control group consisted of

17 patients who are not carriers of mutations of genes of hemostasis.
In addition, patient of the main group were divided into subgroups: subgroup A-18 female patients who are carriers of multigenic thrombophilia, subgroup B - 18 patients - carriers of homozygote mutations of MTHFR, subgroup C - 25 patients - carriers of heterozygote mutations of MTHFR.
The biochemical markers of chromosomal pathology (PAPP - A, β-HCG) of the patient serum in the 11-13 weeks of pregnancy were examined. Their values were estimated in MoM, which were used later to calculate the risk of chromosomal pathology by the program "ALFA", UK. In the third trimester (30 weeks to term) the concentration of estriol and placental lactogen were evaluated, a value estimated in nmol / l and mg / L, respectively. Reduction concentrations of the investigated markers was ascertained with a decrease in PAPP-A and hCG less than 0.5 IOM, estriol - by 30-50% of the standard for gestational indices, placental lactogen – less than average meaning for term of pregnancy.

Results

In patients I group concentrations of serum markers in first trimester (PAPP-A, β-HCG) less than 0,5 MoM noted in 8 and 10 cases respectively, while in the control group similar changes were reported only in 0 and 3 patients respectively.
In the third trimester estriol was more variable indicator, reducing the concentration of which was recorded in 5 patients of the main group, while in the control group it is not significantly reduced. Concentrations of placental lactogen was reduced only in 1 patient of main group.

Conclusions

In pregnant women with hereditary thrombophilia in the study of biochemical markers of the first trimester (PAPP-A and β-HCG) significantly more often found their decreasing below 0.5 MoM, including simultaneous, compared with patients without mutations in the genes system of hemostasis. The examination has revealed that there was a decrease of estriol level in the third trimester among the patients in the main group compared to the controls.
The most significant changes in feto-placental complex hormones in all stages of pregnancy are found in patients with multigenic thrombophilia. For those pregnant women reduction, especially synchronous, level of biochemical markers (PAPP-A, β-HCG) in the

TABLE 1 - CONCENTRATION OF MARKER PROTEINS OF FETO-PLACENTAL COMPLEX REDUCTION IN PATIENTS WITH HEREDITARY THROMBOPHILIA.

Group	Reduction of PAPP-A level, n (%)	Reduction of β-HCG level, n (%)	Combined reduction of PAPP-A and β-HCG, n (%)	Reduction of estriol level, n (%)	Reduction of prolactin level n(%)
I (N61)	8(13)	10(16,3)	5(8,2)	5(8,2)	1 (1,6)
II (N17)	0	3(18)	0	0	0

TABLE 2 - CONCENTRATION OF MARKER PROTEINS OF FETO-PLACENTAL COMPLEX REDUCTION IN PATIENTS WITH DIFFERENT GENETIC VARIANTS OF THROMBOPHILIA.

Subgroup	Reduction of PAPP-A level, n (%)	Reduction of β-HCG level, n (%)	Reduction of estriol level, n (%)	Reduction of prolactin level n (%)
A (N18)	3(16,5)	3(16,5)	2(11)	0
B (N18)	3(16,5)	4(22)	2(11)	1(5,5)
C (N25)	2(9)	3(13,5)	1(4,5)	0

TABLE 3 - MEAN VALUES OF PROTEIN MARKERS OF FETO-PLACENTAL COMPLEX IN PREGNANT WOMEN WITH HEREDITARY THROMBOPHILIA.

Subgroup, Group	PAPP-A level, MoM	β-HCG level, MoM	Estriol level, ng/l	Prolactin level mg/l
A (N18)	1,03	0,84	12,6	3,5
B (N18)	1,05	1,3	23,9	4,4
C (N25)	1,59	1,54	22,7	4,3
Group II (N17)	1,6	1,62	28,8	5.32

first trimester may be predictor of later obstetric complications that require additional examination, monitoring and, if necessary, treatment.

In patients with homozygote mutation of gene MTHFR, which has a higher risk of thrombophilia in the third trimester, the most important are markers of the first trimester, reduction level PAPP-A, β-HCG below 0.5 MoM can also serve as a basis for further monitoring of coagulation as well as a feto-placental complex.

There were no significant deviations in marker FPC compared with the control group in patients with heterozygote mutation of gene MTHFR, which may indirectly indicate a lack the risk of thrombophilia and, as a result, feto-platsentarnoy dysfunction that leads to later obstetric complications.

More sensitive and, importantly, early markers of hereditary thrombophilia and as a consequence, various obstetric complications are indicators I trimester PAPP-A and β-HCG

The data continue to accumulate and be processed because, despite numerous studies now have not had the unequivocal scientific conclusion about the possibility of using biochemical markers for the prediction of feto-placental dysfunction. These data require clarification and systematization for the formulation and development of clear prognostic criteria of obstetric complications.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Causes of male infertility factor of a married couple

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Objective

Determining the male factors in infertile marriage. Sterilitas may be diagnosed when one woman is'nt pregnant after one year with not protective intercourse; 1/3 of couple arrived conception after three months of married, and 80% arrived success in the first year of married.
Infertility is termed primary when it occurs without any prior pregnancy, and secondary when it follows a previous conception.
Male factor is present in 30-40% of couples, when 12-15% of sterile married are steril without its hope.
Here is present: Defect of spermatogeneses, anomalies of genital male tract.
Female factor is present in 40-50%. There are many factor which is: infection of cervix uteri, vaginitis, uterin factor, endocrinology factor.
Idiopatic factor is present in 5-10% of cases.

Aims

1. Verification of male factor in infertile couple.
2. Analysis of social factors.

Methodology

We prospectively studied history in the period January 2011-December 2011.

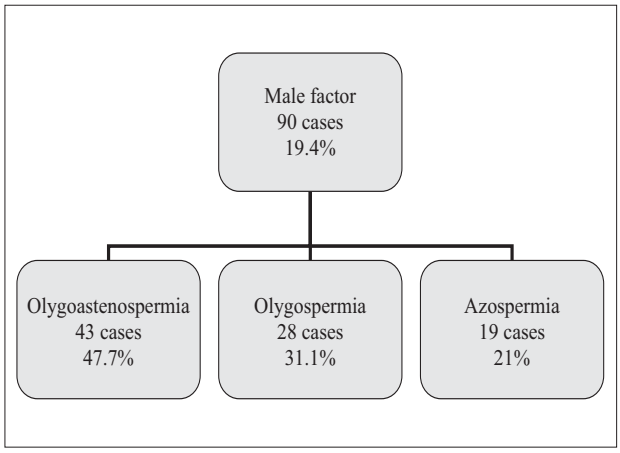
Results

On the 1000 cases, 463 or 46.3% were diagnosed in the group of infertile patients. Of these, 90 (19.4%) as a cause of male infertility (Tables 1 and 2) on this marriages. Treatmant of sterile couple: spermiogram, uterin–tubal factor, cervical factor, ovulation, hormonal status, endometrial biopsy, laparaskopy.

TABLE 1

Spermiogram analysis	Nr of cases	%
Olygoastenospermia	43	47.7%
Olygospermia	28	31.1%
Azospermia	19	21.1%
Total	90	99.9%

TABLE 2



Conclusion

The high percentage of decreased male fertility ability in sterile marriages indicates that in the diagnostic procedure and treatment both partners should be subject to procedures for research, and determine which of the partners is the cause.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Screening and management of bacterial vaginosis in pregnancy

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Objective and methods

To review the evidence and provide recommendations on screening for and management of bacterial vaginoses (BV) in pregnancy.
Examined 286 women were divided into three research groups. We have on the first group included 102 women with clinical signs of abortion in the second group as ab habitualis 89 women, 95 women and group checks (Table 1). This study is prospective and was conducted in Obstetrics Gynecology Clinic in Pristina in the period januar 2011-december 2011. We analyze vaginal swabs of 286 pregnant women. Obtained vaginal swabs are processed in the Microbiology department of the National Institute of Public Health Institute in Pristina. The results obtained were subjected to statistical analysis.
Evaluation include: antibiotics treatment efficacy and care rates, and the influence of the treatment of bacterial vaginosis on the rates of the advance pregnancy outcomes such as preterm labour and delivery and preterm premature rupture of membranes.
Bacterial vaginosis may be diagnosted when one woman have more vaginal discharge.
The presence of BV heightens a nonpregnant womans risk for pelvic inflammatory disease (PID) postoperative infections and HIV transmission, and the presence of BV in pregnant woman may be caused of abort in early pregnancy and preterm labory.
History: Pruritus burning, malodorous discharge (worsened during menses) after intercourse.

Aims

1. Verification of the causes of abortion (Ab) with BV in early pregnancy.
2. Role of iron in patients with BV.
3. Analysis of social factors.
4. Analysis of vaginal discharge in pregnant woman.
5. Influence of bacterial vaginosis in imminent abortion.
6. Influence of bacterial vaginosis in habitual abortion.

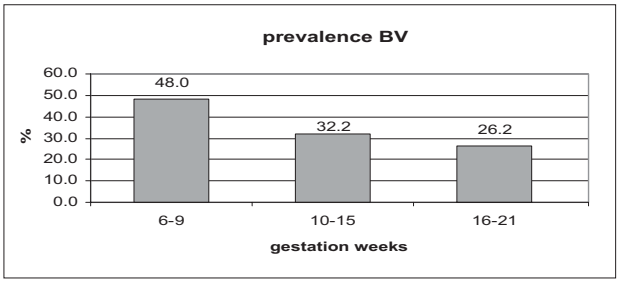
Results

According week gestation with the highest prevalence of bacterial vaginosis was found in week 6-9, with 24 cases or 48.0% prevalence, but was lower in recent weeks gestacion.This changes were statistical significant. (P <0,05) (Table 2).

TABLE 1.

Gestation week	Ab imminens		Ab habitualis		Normal pregnancy		Total	
	N	%	N	%	N	%	N	%
-5	3	2.9		0.0		0.0	3	1.0
6-9	25	24.5	17	19.1	8	8.4	50	17.5
10-15	43	42.2	51	57.3	21	22.1	115	40.2
16-21	20	19.6	17	19.1	47	49.5	84	29.4
>22	11	10.8	4	4.5	19	20.0	34	11.9
Total	102	100.0	89	100.0	95	100.0	286	100.0

TABLE 2.



Bacterial vaginosis are associated with other microrganisms: in 21.7% of cases have been with *E. coli* with prevalence was 13.8%, while 8.0% *Enterococcus* presence (Table 3). So the presence of these bacteria may trigger the search for bacterial vaginosis.

TABLE 3.

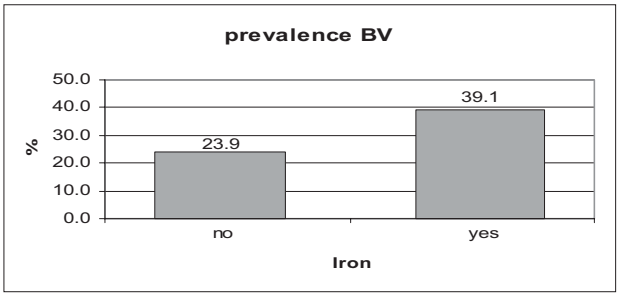
	Ab imminens		Ab habitualis		Normal pregnancy		Total	
	N	%	N	%	N	%	N	%
Candida species	27	26.5	16	18.0	17	17.9	60	21.0
Escheria coli	12	11.8	12	13.5	5	5.3	29	10.1
Enterococcus	3	2.9	16	18.0	6	6.3	25	8.7
Others	8	7.8	7	7.9	7	7.4	22	7.7
Total	102	100.0	89	100.0	95	100.0	286	100.0

The use of iron has affected level of bacterial vaginosis, which has been higher in pregnant who have used iron 39.1% compared with those who have not used 23.9%. These differences have been with statistical values, ($p < 0.05$) (Tables 4 and 5).

TABLE 4.

Iron	Ab imminens		Ab habitualis		Normal pregnancy		Total	
	N	%	N	%	N	%	N	%
No	53	52.0	34	38.2	30	31.6	117	40.9
Yes	49	48.0	55	61.8	65	68.4	169	59.1
Total	102	100.0	89	100.0	95	100.0	286	100.0

TABLE 5.



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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Lipoxin A_4 decreases lesion volume and reduces inflammatory and angiogenic mediators in a mouse model of endometriosis

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Background

Endometriosis affects approximately 10% of women of reproductive age and is associated with inflammation and increased estrogen production.
The main clinical features are chronic pelvic pain, pain during intercourse and infertility.
The mechanisms by which endometrial tissues attach, proliferate, avoid immune surveillance and derive a local vasculature at ectopic sites are not well understood but include dysregulated estrogen and pro-inflammatory cytokine production and activity.
The medical treatment of endometriosis is limited due to high costs, adverse side effects and chances of recurrence after discontinuation of therapy.
Thus there is an unmet need for new drugs which inhibit the progression of endometriosis and alleviate pain and infertility, without affecting ovulation. The aim of this study was to evaluate the effects of the eicosanoid Lipoxin A_4 (LXA_4), which exhibits both anti-inflammatory and pro-resolving properties, in a mouse model of endometriosis.

Material and methods

Endometriosis was surgically induced in 8-9 week old wild type female C57/BL6 mice. 10 mice per group received either vehicle or 5µg/kg LXA_4 a day for 21 days by intraperitoneal injection.

Results

A significant ($p<0.01$) reduction in lesion volume was observed in the LXA_4-treated group ($1.2\pm0.2mm^3$) vs. vehicle-treated mice ($2.6\pm0.36\ mm^3$). The anti-inflammatory effects of LXA_4 included atteutation of IL-1β mRNA expression (100%±6.0 vs. 54±3.5). LXA_4 also attenuated the expression of HIF-1α (100±4.2 vs. 70±5.2) & its target genes COX2 (100±5.3 vs. 61.3±2.0) & VEGF at mRNA (100±2.8 vs. 62±2.2), thus displaying anti-angiogenic effects. VEGF levels in peritoneal lavages were reduced in LXA_4-treated (5±0.95pg) compared to vehicle-treated mice (11±0.90pg) VEGF/50µg of total protein ($p<0.0001$). LXA_4 also reduced the expression of ERα (100±3.6 vs. 64±3.1), cMYC (100±3.8 vs. 62±2.2) and aromatase (100±4.4 vs. 49±2.3) indicating that this lipid may also mediate anti-estrogenic and anti-proliferative activities. These novel findings provide an insight into mechanisms underlying the decreased lesion volume observed upon LXA_4 treatment (data shown are % expression mean ± SEM, where expression in lesions of vehicle-treated mice is normalized to 100% expression vs. expression in lesions of LXA4-treated mice, p value<0.0001 for all genes mentioned above).

Conclusions

Taken together these findings indicate that LXA_4 could be a possible therapeutic for peritoneal endometriosis.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Serum myeloperoxidase and adenosine deaminase activities in polycystic ovary syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in reproductive age women, affecting approximately 6.5-8% (1,2). PCOS is a multifactorial disease and its exact cause remains a mystery.
Inflammation is thought to play a role in the pathogenesis of PCOS (3-8). Myeloperoxidase (MPO) is a peroxidase enzyme released by azurophilic granules of activated neutrophils and macrophages and produces hypochlorous acid (HOCl) and chloride anion (Cl) (9). HOCl, which is an oxidizing species leading to oxidative tissue damage, plays a role in the pathogenesis of atherosclerosis. Thereby, it has been found to be related to cardiovascular disease (10-12).
Adenosine deaminase (ADA) is a cytosolic enzyme found particularly in lymphoid tissue and is increased in immune mediated diseases such as rheumatoid arthritis, familial Mediterranean fever, and tuberculosis (13-15). ADA may also be attributed to inflammation related to coronary heart disease (CHD) (16).
We have not encountered any study evaluating serum MPO and ADA activities in women with PCOS in the literature. In the present study, we aimed to investigate the role of MPO and ADA activities in PCOS. We also aimed to study whether there was a correlation between ADA, MPO, sex hormones, and lipids in these patients.

Material and methods

Patients and setting
The study was conducted with 45 consecutive patients with PCOS (study group) and 40 age- and body mass index (BMI)-matched healthy women (control group) from the outpatient clinics of the Obstetrics and Gynecology Department of Yuzuncu Yil University Hospital, Van, Turkey.
The diagnosis of PCOS was based on the Rotterdam criteria (17). The subjects had not been taking any drugs like insulin sensitizers, oral contraceptives, antiandrogens, glucocorticoids, ovulation induction agents, or other hormonal drugs for at least 6 months before the study. All subjects were informed about the study protocol and written consent was obtained from each. The exclusion criteria included Cushing's syndrome, androgen secreting tumor, diabetes, nonclassical congenital adrenal hyperplasia, thyroid dysfunction, estrogen intake, and hyperprolactinemia. Free androgen index (FAI) was computed according to the following formula (18): Total testosterone level (nmol/l) / sex hormone-binding globulin (SHBG) level (nmol/l) x 100.

Laboratory analysis
After an overnight fast of 12 hours, 10 cc of venous blood was taken from each woman during the early follicular phase (between days 3 and 5) of the spontaneous or progestin-induced menstrual cycle between 8 AM and 10 AM. Then 2 cc was separated to assay MPO and ADA activities. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4°C and were stored at -80°C until analysis. MPO and ADA were analyzed at the Department of Medical Biology of Yuzuncu Yil University.
The serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, and estradiol were assessed by chemiluminescent immunoassay with an Immulite® 2000 an-

alyzer (Diagnostic Products Corp., Los Angeles, CA, USA) and SHBG was analyzed by chemiluminescence microparticle immunoassay (Architect SHBG reagent kit; Biokit, Barcelona, Spain for Abbott Laboratories Diagnostic Division).
Total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were measured by enzymatic colorimetric assay, using a Roche-Hitachi PP Modular Analyzer (Roche-Hitachi, Tokyo, Japan) and its original reagents. High-sensitive CRP (hs-CRP) was determined using immunonephelometric methods and a BN-II analyzer (Dade Behring, Germany). Leukocyte count was determined with an automatic cell counter (Beckman Coulter, Coulter LH 780 Analyzer, USA).
ADA was determined by the method described by Giusti (19) and MPO was assayed by the method described by Bradley (20).

Statistical analysis
Results were expressed as mean and standard deviation (SD). Student's *t*-test was performed to evaluate differences between the group means. Pearson's correlation coefficient was used to evaluate the relationship among the variables, and p values <0.05 were regarded as statistically significant.

Results

The groups were similar with respect to mean age, BMI, and WHR (Table 1). In the PCOS group, total testosterone and FAI were significantly higher in comparison with the control group ($p=0.001$). Serum estradiol level was also significantly elevated in women with PCOS than in the controls ($p<0.05$). Serum FSH and LH levels of PCOS patients were 4.25±1.68 and 9.24±1.86, respectively. Although the women with PCOS had higher hs-CRP, triglycerides, total cholesterol, and LDL cholesterol than the healthy subjects, the difference was not statistically significant ($p>0.05$) (Table 2).
Leukocyte count was significantly higher in women with PCOS than in the control group ($p=0.001$), although leukocytosis was not found in either group. In the leukocyte formula, lymphocytes, monocytes, and neutrophils were significantly higher in the PCOS group than in the controls ($p<0.05$). Although serum MPO activity was significantly higher ($p<0.05$), ADA activity was similar in women with PCOS compared to the controls ($p>0.05$) (Table 3).
There was a significant correlation between serum ADA and hs-CRP level in the PCOS group ($r=0.853$ $p<0.01$) but we did not find any association between serum MPO, ADA level, and serum sex hormones and lipid profiles.

Discussion

In our study, serum total testosterone level and FAI were significantly higher in the PCOS group compared to the controls. Cho et al. showed a higher total testosterone level and FAI in their PCOS group and suggested that FAI was a better marker to distinguish hyperandrogenism in patients with PCOS (21). Serum estradiol level was also found to be significantly higher in women with PCOS than in the controls. Amato et al. showed a low estradiol/testosterone level in women with PCOS and associated it with chronic oligo-anovulation and atherogenic lipid profile (22). Chronic oligo-anovulation might cause high serum estradiol levels in women with PCOS.
There is an interaction between the immune and reproductive systems. In the ovary, migration of leukocytes in and out of the organ is permitted by its vascularization and anatomy. These leukocytes secrete numerous inflammatory substances that play important roles in the cyclic events and some ovarian disorders like PCOS (23). In our study, we found significantly higher leukocyte counts especially in lymphocytes, monocytes, and neutrophils in the PCOS group compared to the controls. After Orio et al. firstly demonstrated an increased leukocyte count in PCOS (4), the relationship between chronic low grade inflammation and PCOS has been clarified with subsequent studies (3,24-30). The results of most studies, including ours, indicate an increased leukocyte count (especially the lymphocytes, monocytes, and neutrophils), supporting the role of inflammation in PCOS pathogenesis.
In the present study, hs-CRP level was higher in the PCOS group compared to the controls but the difference was not statistically significant. In some studies, serum hs-CRP levels of women with PCOS were also similar with the values of healthy controls (31-34). Escobar-Morreale et al. reviewed the studies evaluating the status of serum inflammatory markers in PCOS patients. They concluded that these women had elevated CRP levels in circulation independent of obesity and this result was regarded as evidence of chronic low grade inflammation (35). The difference between the results of the present study and their review may be due to the relatively low number of subjects in our groups. However, with this investigation, a significant correlation between serum CRP and ADA levels has been shown in normal weight women with PCOS for the first time ($p<0.01$). This correlation was not detected in the control group ($p>0.05$).
To the best of our knowledge, this is also the first study investigating serum MPO and ADA activities in women with PCOS. In the present study, serum MPO level was higher in the PCOS group compared to the

controls. Sasıkala et al. indicated that MPO was an excellent inflammatory marker and showed elevated MPO levels in the ovarian and uterine tissues of PCOS induced rats (36). Dursun et al. also found higher MPO levels in gingival cervicular fluid of women with PCOS (37). One of the mechanisms underlying the pathogenesis of PCOS is chronic low grade inflammation and MPO is an enzyme released from activated neutrophils, and macrophages. Therefore, the finding of increased monocyte and neutrophil counts observed in our study is compatible with the elevated serum MPO activity in the women with PCOS. Besides showing inflammation, increased MPO level has also been associated with cardiovascular disease (10,11,16,38).

In conclusion, PCOS pathogenesis may involve an inflammatory process by increasing serum MPO activity independent of sex hormones, body mass index, and lipid profiles. Assessing the usefulness of serum MPO activity to predict the risk of cardiovascular disease in women with PCOS may be a topic for future studies.

TABLE 1 - DEMOGRAPHIC CHARACTERISTICS OF THE PCOS GROUP AND THE CONTROL GROUP.

	PCOS (n = 45)	Controls (n = 40)	*P*
Age (year)	22.82 ± 4.13	24.2 ± 3.32	0.08
BMI (kg/m^2)	22.03 ± 3.38	22.29 ± 3.40	0.434
WHR	0.73 ± 0.09	0.75 ± 0.08	0.481

Note: Results are presented as mean ± SD.
PCOS=Polycystic ovary syndrome, BMI=body mass index, WHR=waist-to-hip ratio.

TABLE 2 - SERUM HORMONE LEVELS OF THE PCOS GROUP AND THE CONTROL GROUP.

	PCOS (n = 45)	Controls (n = 40)	*P*
AFSH (IU/l)	4.25 ± 1.68	5.66 ± 1.20	0.24
LH (IU/l)	9.24 ± 1.86	6.03 ± 0.92	0.13
Total testosterone (ng/dL)	81.86 ± 34.39	40.72 ± 14.42	0.001*
Estradiol (pg/ml)	75.64 ± 56.59	50.39 ± 27.34	0.047*
SHBG (nmol/l)	43.28 ± 4.22	56.39 ± 20.44	0.043*
FAI	6.56 ± 2.72	2.51 ± 1.26	0.001*

Note: Results are presented as mean ± SD.
PCOS=Polycystic ovary syndrome, FSH=follicle-stimulating hormone, LH=luteinizing hormone, SHBG=sex hormone-binding globulin, FAI=free androgen index.
* $p<0.05$ statically significant

TABLE 3 - METABOLIC FEATURES OF THE PCOS GROUP AND THE CONTROL GROUP.

	PCOS (n = 45)	Controls (n = 40)	*P*
Total cholesterol (mg/dl)	178.90 ± 48.82	161.33 ± 31.84	0.274
LDL cholesterol (mg/dl)	100.68 ± 40.51	89.94 ± 25.35	0.414
Triglycerides (mg/dl)	87.97 ± 34.48	84.73 ± 39.74	0.81
WBC count (x10^3/ml)	7.69 ± 2.00	5.58 ± 1.32	0.001*
Neutrophils (x10^3/ml)	4.79 ± 1.83	3.32 ± 1.37	0.002*
Monocytes (x10^3/ml)	0.50 ± 0.18	0.38 ± 0.17	0.018*
Lymphocytes (x10^3/ml)	2.22 ± 0.63	1.79 ± 0.59	0.012*
MPO (IU/l)	36.19 ± 10.38	30.24 ± 8.74	0.02*
ADA (IU/l)	23.79 ± 7.34	22.70 ± 6.77	0.551

Note: Results are presented as mean ± SD. PCOS=Polycystic ovary syndrome, LDL=low-density lipoprotein, WBC=white blood cell, MPO=myeloperoxidase, ADA=adenosine deaminase.
* $p<0.05$ statically significant

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Evaluation of the relationship between endogenous gonadotropins and female sexual function and psychological status in predialysis and hemodialysis patients

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Introduction

Sexual dysfunction and depression are common problems in patients with chronic renal failure (CRF) (1,2). Approximately 75% of women undergoing hemodialysis treatment have sexual problems (3). Women with end-stage renal disease describe a decrease in sexual function (4).
In these patients, psychological factors also play a role in the mechanism of sexual dysfunction in addition to the hormonal changes (5). The prevalence of depression in hemodialysis patients ranges from 41.7% to 58% (6,7). Sexual dysfunction as a result of biological factors may also lead to depression. Therefore, sexual dysfunction and depression create a vicious circle in these patients (8). Thus, the patients with CRF should also be managed for psychosocial well-being and sexual life in addition to renal care.
We searched Pubmed in April 2011 using the keywords "female sexual dysfunction", "depression", "hemodialysis", and "Turkey" and found only 3 studies (8-10). However, in all of these studies, patients with end-stage renal disease had been evaluated. We have not seen any study evaluating both sexual dysfunction and depression in female predialysis patients. Thus, we aimed to investigate sexual function and psychological status and the relation between sexual dysfunction and depression, demographic features, and hormonal parameters in predialysis and hemodialysis patients.

Materials and methods

The study was conducted with 77 female subjects, comprising 22 predialysis patients, 25 hemodialysis patients, and 30 controls (Table 1). The predialysis group consisted of randomly selected patients with stage IV chronic kidney disease (glomerular filtration rate (GFR) = 15–29 ml/min/1.73 m^2). The hemodialysis group was composed of patients receiving hemodialysis treatment, three times a week, 4 hours for one dialysis procedure for at least 6 months at the Hemodialysis Center of Yuzuncu Yil University, School of Medicine, Van, Turkey. Healthy female volunteers were included as controls. This study was approved by the Ethical Committee of the university and informed consent was obtained from all subjects. The eligibility criteria included: age between 18 and 65 years, female gender, married, sexually active, no psychiatric treatment in the previous 6 months, and the intellectual and mental capacity to understand and answer the questionnaire. Exclusion criteria were being younger than 18 years or older than 65 years, being on chronic dialysis for less than 6 months (for hemodialysis patients), having a psychiatric disease or infection, or having undergone surgical menopause.
After an overnight fast of 12 h, a total of 10 cc of venous blood was taken from each woman between 8 AM and 10 AM (for hemodialysis patients, before the dialysis). The serum concentrations of glucose, creatinine, phosphorous, calcium, parathyroid hormone (PTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone, estradiol, and hemoglobin were assessed in all groups. Sexual functioning was evaluated by the Arizona Sexual Experiences Scale (ASEX). A total ASEX score ≥19, any one item with a score ≥5, or any three items with a score of 4 have all been found to be correlated with sexual dysfunction (11). The reliability and validity

study of the Turkish version of ASEX had already been demonstrated by Soykan (12).
Depression was assessed by the Beck Depression Inventory (BDI). Its 21 items were scored from 0 to 3. Each item was rated with a four-point Likert system (13). The BDI was adapted for Turkey by Hisli (14,15). Subjects who had a score over 16 were classified as depressed.

Statistical analysis
The patients' characteristics were expressed as mean ± standard deviation (SD). When the groups were unequal and nonparametric, the data were analyzed using the Kruskal-Wallis test. Comparing more than two groups, one-way ANOVA test and then Duncan's method for pair-wise comparison were used for equal and parametric groups. Pearson's correlation analysis was used to determine the relationships among the variables. Odds ratios were calculated to determine the effect of depression on sexual dysfunction. A p value <0.05 was regarded as statistically significant.

Results

The mean serum concentrations of PTH, FSH, and LH were higher but estradiol and hemoglobin levels were lower in the predialysis and hemodialysis groups than in the controls (Table 2).
The patients in the predialysis and hemodialysis groups had higher mean BDI, ability to reach orgasm, and total ASEX scores than the controls (p <0.05) (Table 3). Regarding arousal, erection/lubrication, and satisfaction with orgasm, no differences were found among the groups (p >0.05). The mean score for drive was higher in the hemodialysis group than in the control group (p = 0.04). The patients in the predialysis group were 6 (odds ratio: 6.00, CI: 1.68–21.48) and 3.8 times (odds ratio: 3.8, CI: 1.09–13.18) more likely to develop depressive symptoms compared to the controls and hemodialysis patients, respectively. In the hemodialysis group, the patients with and without depression did not show any significant difference with respect to the development of sexual dysfunction (p = 0.126). However, the patients with depression were 24 times more likely to develop sexual dysfunction compared to the patients without depression in the predialysis group (odds ratio: 24 CI: 2.06–27.49). In the predialysis group, age and mean serum FSH and LH levels were positively correlated with arousal and erection/lubrication scores (p <0.05). BDI score was positively correlated with total ASEX score (r = 0.474, p <0.05) in the predialysis group and with satisfaction with orgasm score (r = 0.563, p <0.01) in the hemodialysis group but we did not find any correlations between BDI score and gonadotropins or other hormones (Table 4).

Discussion

Decreased libido and reduced ability to reach orgasm are common problems in women on dialysis (16,17). However, there are few studies about female sexual dysfunction in predialysis patients. Basok et al. showed reduced desire, arousal, orgasm, and satisfaction in predialysis patients compared to controls (18). In our study, total ASEX score was higher in predialysis and hemodialysis patients compared to the controls, reflecting impaired sexual function (p = 0.016). We found reduced ability to reach orgasm in both the predialysis and hemodialysis groups. We also noted that sexual desire was significantly lower in the hemodialysis group compared to the predialysis and control groups (p = 0.04). Therefore, we may conclude that hemodialysis treatment reduces sexual desire and leads to sexual failure.
The occurrence of sexual dysfunction is multifactorial. Berman et al. showed that older women and menopausal women who did not use hormone replacement therapy had significantly lower physiologic response by sexual stimulation (19). Peng et al. noted that sexual dysfunction was common in female hemodialysis patients and they found strong relation between sexual dysfunction and increasing age and depression (20). Oksuz et al. and Cayan et al. also showed increasing in sexual dysfunction with age (21,22). In our study, we found a significantly positive correlation between age and arousal, erection/lubrication, and total ASEX score in predialysis patients. Increasing age may be related to vascular insufficiency, decreased estrogen concentration in circulation, decreased blood flow and function of urogenital organs, and changes in body image.
Anemia is associated with sexual dysfunction in patients with end stage renal disease (23,24). In our study, we did not find any correlation between ASEX scores and hemoglobin levels. This may have resulted from the low number of patients in our study.
Anovulatory cycles are frequent in uremic women (25). The features of uremic hypogonadism in women were composed of elevated gonadotropins and prolactin and decreased estradiol levels (26,27). Disturbances in reproductive hormones may impair hypothalamic-pituitary function and lead to sexual dysfunction (1,18). In the present study, we found higher serum PTH, FSH, and LH and lower estradiol levels in the predialysis and hemodialysis groups than in the control group. In addition to hypothalamic-pituitary dysfunction, vaginal dryness and atrophy due to the reduced estrogen concentration may cause decreased lubrication and discomfort during intercourse.
Besides hormones, psychological factors also influence

the sexual life in women with CRF [20,28,29]. Depression rates ranged from 24.1% to 58% in women with CRF (7,30). We noted depression in 54.5% and 24% of predialysis and hemodialysis women, respectively. We also found that predialysis patients were 6 and 3.8 times more likely to develop depression compared to the patients in the control and hemodialysis groups, respectively. This may be associated with the lack of behavioral compliance in predialysis patients before dialysis treatment. These patients may be anxious due to uncertainty about their health after this and so this situation may result in stress and cause depression. We also found that sexual dysfunction was up to 24 times more common in depressive women in the predialysis group.

In conclusion, female predialysis patients seem to be more likely to have depression and those with depressive symptoms may be at higher risk of developing sexual dysfunction. In these patients, increased gonadotrophin levels and age may be the other contributing risk factors for developing this sexual dysfunction. Therefore, prompt gynecologic and psychiatric evaluation of female predialysis patients in terms of their psychological and sexual aspects might be advisable. Further studies with higher numbers of patients are needed to obtain more information on this topic.

TABLE 1 - DEMOGRAPHIC CHARACTERISTICS OF PREDIALYSIS, HEMODIALYSIS AND CONTROL GROUPS.

	Predialysis (n=22)	Hemodialysis (n=25)	Control (n=30)	*P*
Age (year)	43.68 ± 7.99	45.04 ± 10.58	42.57 ± 10.84	0.56
BMI (kg/m^2)	26.61 ± 5.40	24.86 ± 5.99	25.15 ± 3.90	0.54
Gravidity (number)	8.84 ± 4.34 [a]	7.32 ± 3.17 [b]	3.92 ± 3.71	0.001*
Duration of CRF (month)	46.09 ± 44.99	52.92 ± 37.36	–	0.57
Duration of dialysis (month)	–	41.36 ± 35.10	–	
Education (%)				0.01*
None	19 (86.4%) [a]	18 (72%) [b]	11 (36.7%)	0.005*
Primary school	3 (13.6%) [a]	7 (28%)	11 (36.7%)	0.04*
High school	–	–	4 (13.3%)	
University	–	–	4 (13.3%)	
Occupational status (%)				0.11
Unemployed	22 (100%)	24 (96%)	25 (83.3%)	
Employed	–	1 (4%)	5 (16.7)	
Monthly income (won)				0.23
<634 $	10 (45.5%)	12 (48%)	8 (26.7%)	
>634 $	12 (54.5%)	13(52%)	22 (73.3)	

BMI=body mass index, CRF=chronic renal failure, $=United States Dollars
*p<0.05, [a] predialysis versus control, [b] hemodialysis versus control.

TABLE 2 - COMPARISON OF BIOCHEMICAL, HORMONAL AND CLINICAL CHARACTERISTICS OF PREDIALYSIS, HEMODIALYSIS AND CONTROL GROUPS.

	Predialysis (n=22)	Hemodialysis (n=25)	Control (n=30)	*P*
Glucose (mg/dl)	95 ± 14.13	93.23 ± 11.47	91.07 ± 9.31	0.48
Creatinine (mg/dl)	3.05 ± 1.56 [a]	7.15 ± 2.63 [b,c]	0.65 ± 0.13	0.001*
Hemoglobin (g/dl)	10.57 ± 1.66 [a]	10.91 ± 1.90 [b]	13.18 ± 2.84	0.003*
Serum phosphorous (mg/dl)	4.15 ± 0.83	5.33 ± 2.25 [b,c]	3.43 ± 0.55	0.001*
Total serum calcium (mg/dl)	8.82 ± 0.67 [a]	8.57 ± 0.97 [b]	9.24 ± 0.50	0.004*
Intact PTH (pg/ml)	265.05 ± 253.64 [a]	358.33 ± 317.36 [b]	60 ± 26.03	0.001*
Single-pool Kt/V	–	1.43 ± 0.23	–	
FSH (mIU/ml)	53.68 ± 9.43 [a]	43.45 ± 11.04 [b]	15.30 ± 4.27	0.003*
LH (mIU/ml)	36.19 ± 6.64 [a]	39.43 ± 9.54 [b]	8.52 ± 1.68	0.001*
Prolactin (mIU/ml)	18.88 ± 15.76	42.69 ± 24.94 [b,c]	18.19 ± 14.66	0.001*
Total testosterone (ng/ml)	0.68 ± 0.25	0.65 ± 0.22	0.69 ± 0.20	0.06
Estradiol (pg/ml)	45.81 ± 13.09 [a]	48.38 ± 10.73 [b]	81.52 ± 14.30	0.03*

PTH=parathyroid hormone, FSH=follicle-stimulating hormone, LH=luteinizing hormone.
[a] predialysis versus control
[b] hemodialysis versus control
[c] predialysis versus hemodialysis

TABLE 3 - THE COMPARISON OF MEAN ASEX AND BDI SCORES OF THE WOMEN IN PREDIALYSIS, HEMODIALYSIS AND CONTROL GROUPS.

	Predialysis (n=22) Mean ± SD	Hemodialysis (n=25) Mean ± SD	Control (n=30) Mean ± SD	*P*
BDI score	16.05± 7.66 [a]	14.28 ± 8.09 [b]	8.03 ± 7.52	0.001*
Drive	3.71 ± 1.76	4.04 ± 1.67 [b]	3 ± 1.17	0.04*
Arousal	4.14± 0.96	3.8 ± 1.50	3.33 ± 1.15	0.07
Erection/lubrication	4 ± 1.14	3.32 ± 1.68	3.17 ± 1.23	0.095
Ability to reach orgasm	4.33± 0.80 [a]	3.96 ± 1.49 [b]	3.27 ± 0.98	0.004*
Satisfaction with orgasm	3.48 ± 1.47	3.52 ± 1.23	3.03 ± 1.22	0.311
Total ASEX score	19.67 ± 4.26 [a]	18.64 ± 5.89 [b]	15.8 ± 4.37	0.016*

p<0.05
ASEX=Arizona Sexual Experiences Scale, BDI=Beck Depression Inventory.
[a] predialysis versus control
[b] hemodialysis versus control

TABLE 4 - THE CORRELATION COEFFICIENTS BETWEEN TOTAL ASEX SCORES, BIOCHEMICAL AND DEMOGRAPHICAL CHARACTERISTICS OF THE PATIENTS IN PREDIALYSIS AND HEMODIALYSIS GROUPS.

	Age		FSH		LH		BDI	
	PreD	D	PreD	HD	PreD	HD	PreD	HD
Drive	0.296	0.335	0.028	0.038	0.181	0.113	0.24	0.116
Arousal	0.521*	0.022	0.653*	0.351	0.498*	0.377	0.398	0.228
Erection/ lubrication	0.463*	0.025	0.555*	0.398	0.587*	0.347	0.322	0.159
Ability to reach orgasm	0.33	0.011	0.555*	0.350	0.357	0.338*	0.407	0.24
Satisfaction with orgasm	0.284	0.389	0.158	0.153	0.028	0.135	0.354	0.563**
Total ASEX score	0.524*	0.191	0.466*	0.393	0.271	0.356	0.474*	0.314

*p<0.05; **p<0.01
ASEX: Arizona Sexual Experiences Scale, FSH: follicle-stimulating hormone, LH: luteinizing hormone, BDI: Beck Depression Inventory, PreD: Predialysis, HD: Hemodialysis.

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Assessing the glucocorticoid therapy as a significant risk factor in postmenopausal bone evaluation

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Introduction

Several risk factor must be taken into consideration approaching the prevention of osteoporosis in postmenopausal subjects such as low body weight, maternal history of fractures, falls and direction of falls, previous long period of oligo-amenorrea and some secondary causes of osteoporosis. The well known main cause of iatrogenic osteoporosis is the glucocorticoid therapy (1). In recent decades it has been registered a widespread increasing use of synthetic GCs for the treatment of autoimmune, reumathologic, pulmonary and gastrointestinal disorders. The GC use is frequent in aging people but is growing the use of these drugs in several diseases in young women too often during the transitional menopausal period. GC-induced osteoporosis, alike the postmenopausal osteoporosis, mainly affects the trabecular bone determining a BMD quick fall as a result of excessive bone resorption (2) this leads to an increased risk of fractures which may occur even few months after the start of the therapy (3).
In 60-year or older postmenopausal women decreased levels of estradiol are associated with a substantial increase of fractures (4) while during the early menopause even the osteopenia might be associated to fractures especially if combined with multiple risk factors.
The aim of the study was to analyze the potential effects and consequences of CG treatment without any preventing treatment in an outpatient cohort of subjects who were referring to their physicians for a follow-up visit in comparison with a large sample of healthy postmenopausal women. Therefore the purpose of our investigation was to highlight the importance of the G-C therapy in the female population as a decisive risk factor for osteoporosis and fractures if associated with early postmenopausal period.

Materials and methods

Adult patients attending the multicenter cohort study EGEO (GC and Osteoporosis Epidemiology) organized by Lazio GISMO group (Italian Group for Diagnosis of Bone Metabolism Diseases) were interviewed and submitted to an established questionnaire. The EGEO study was designed with the aim to study the local epidemiology of the glucocorticoid-induced osteoporosis (GIO) and involved patients taking long term GC therapy. At the same time even patients attending the centre for menopause and osteoporosis of our institution between February 2009 and November 2010 were interviewed and submitted to the same questionnaire. Three hundred and fifty one women were enrolled. 192 patients having had prescription of glucocorticoids for different purpose were compared with 139 postmenopausal patients and 20 premenopausal woman. No subjects had hormonal replacement therapy or antiosteoporotic therapy. Menopausal patients were women who had experienced amenorrhea for over 12 months. All patients had received a diagnosis of osteopenia or osteoporosis in the lumbar spine (L1-L4) or femur by DXA Lunar-DPX or Hologic according the 1994 WHO criteria. No patients had previous GC treatments.
Data were recorded and provided information about age, BMI, family fractures history, spontaneous or consequent to falls from standing position fractures, numbers and sites of fractures, the most

recent bone mineral density available test (preferably the Dual-Energy-X-Ray Absorptiometry (DXA). Therefore information about glucocorticoid therapy, type of molecule, dosage and duration of therapy as well as type of antiresorptive drugs and/or calcium and vitamin D supplementation and hormonal therapy were collected. All patients were interviewed by trained dedicated personnel. Reported fractures have been assessed by radiological diagnosis.

Statistical analysis

Quantitative variables were tested for normal distribution and compared by means of two-tailed t-test. Differences in groups were assessed by use of the $\chi 2$ test and Fisher's exact test. Data through the manuscript are expressed as mean $\pm$ SD. A P value less than 0.05 was considered statistically significant.

Results

Three hundred and fifty one patients were included in the study: 192 female patients who had GC treatment for longer than 3 months without any anti-osteoporotic treatment (22 patients were premenopausal and 170 postmenopausal), 139 control postmenopausal and 20 premenopausal women. No patients had hormonal therapy or other treatment for osteoporosis.

Table 1 - Depicts the demographic and clinical features of GC-treated patients and of healthy control patients. The two groups didn't differ for age, BMI and family history of falls

Table 2 - Shows clinical and densitometric data in GC-treated patients and in pre e postmenopausal subjects according to the age.

Among the GC treated subjects under 50 years the percentage of osteopenia /osteoporosis was significantly higher than in the premenopausal subjects of correspondent-age (P<0.001). Menopausal patients between 50 and 65 years showed a significant higer mean T score than GC treated patients. Therefore in spite of a similar percentage of normal T score in the two groups and a significant higher percentage of osteopenia in menopausal subjects (58,5% vs 32.9% p<0.01) the percentage of ostoporosis among GC treated patients was significantly higher (30% vs 7.3% 0.001). In the older menopausal population (over 65 years) the prevalence of osteopenia was hardly higher in GC treated patients but the osteoporosis prevalence was similar.

The distribution of fractures among age-selected groups was illustrated in the Figure 1.

Fragility fractures occurs in GC treated patientswith a doubled or tripled frequencies in comparison with control subjects.

TABLE 1.

	GC treated	Control woman
Age	63,7±11	68,2±14
BMI	25,2±4,5	26,2±5
Family hystory of falls)	22%	20%
Duration of therapy	3-6 months 12% 6-12 month 16% > 12 month 72%	
Type of disease treated	Rheumatologic 62% Pneumatologic 22% Dermatologic 4% Other 12%	

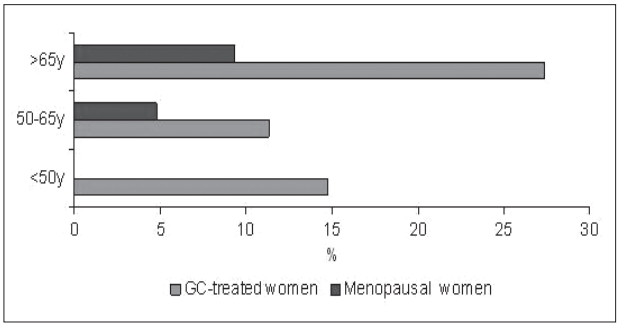

Fig. 1 - Percentage of fragility fractures.

TABLE 2.

	GC treated patients			Menopausal subjects		
				premenopausal	postmenopausal	
	<50 years 22 pts	50-65 years 79 pts	>65years 91 pts	<50 years 20 pts	50-65 years 58 pts	>65years 61 pts
Age	43±2	58,7±4,2	72,5±5,3	46±3,5	56,8±4,4	69,8±3,2
BMI	22,7±3	24,2± 3,5	24,4± 3,5	25,3±4	26,8±3,9	27,2±5
T score L1-L4 (DEXA)	-2,05±1,2	-2,3±1	-2,5±1	-0,63±1	-1,1±1	-1,8±0,8
Osteopenia %	37%	32,9%	47,3%	11%	58,5%	31,8%
Osteoporosis %	22,2%	30,3%	40,6%	0%	7,3%	38%

Discussion

The use of GC in the treatment of autoimmune, pulmonary, and gastrointestinal rheumatologic disorders has been increasing in the last decade (2,5-8). Despite the knowledge of the deleterious effects on skeletal bone, till now few patients receiving GC therapy are considered at risk for bone loss. Indeed our sample of GC treated patients who haven't had any preventing treatment coming from the observational regional study EGEO represent the 45% of the entire female cohort. The modest attention to the GIO prevention brings about serious consequences in the menopausal population.

Several mechanisms involved in bone loss induced by GC therapy are well known (2): many of them cause changes of bone metabolism similar to those induced by estrogen loss, so that there might be likeness between two types of osteoporosis while some differences must be significant.

In patients GC treated the loss of bone mineral density is biphasic; a first phase, within the first years of treatment, determines a loss of 6-12% and a second phase showing a reduction of approximately 3% yearly (1). The postmenopausal bone density reduction is estimated to be about 0.5-3% yearly with higher bone loss in the early period of menopause (9).

The effects of GC therapy in young population appear to be really deleterious. Our young GC-treated population (consisting in 22 premenopausal women and 5 early menopausal subjects) shows a worrying low mean vertebral T-score with a significant higher percentage of osteoporosis in comparison with the corresponding menopausal population (aged from 30 to 50, with 4 subjects with early menopause less than 40years). Particularly the incidence of fractures was very high confirming the fact that GC treatment worsens the bone strength affecting not only the bone density but even the bone quality.

The risk of fracture was found to increase with doses of drugs and length of therapy therefore patients who were precociously GC treated and hadn't prophylactic measures at the menopausal period are at special risk of fractures.

Our data show that in the GC-treated menopausal population the prevalence of osteoporosis was significantly higher than in control menopausal subjects. The incidence of vertebral and non vertebral fractures was almost doubled or tripled in over 65 GC treated patients. Considering the istomorphometric features several studies showed that osteoporosis GC induced is characterized by fewer osteoblasts and an increased prevalence of osteocyte apoptosis (10). Glucocorticoids excess directly reduces osteoclast production even if the lifespan is prolonged. Therefore, with long-term therapy, the number of osteoblasts is reduced as well as the bone formation (11) consequently the bone strength is deeply compromized. The estrogen deficiency is associated with an increase in lifespan of osteoclasts and characterized by concomitant decrease in osteoblasts action and lifespan (12). These effects are also accentuated by the preresorptive action of iperPTHrp (13) resulting in an extensive calcium mobilization.

Fractures decline after discontinuation of GC-therapy (3) while patients becoming osteopenic or osteoporotic approaching menopause, according to our data, the fracture increases the risk of fracture.

This study highlighted the idea that in GC-treated patients (above all if they haven't a right preventive therapy) keep a damage in the skeletal structure which is increased with an additive effect by menopause.

The importance of this study is the precise assessment of osteoporosis risk factors which may help to guide any early intervention.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The vanishing pituitary mass

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Case report

A 34-year old female, with no background medical history, presented with nocturia, polyuria, polydipsia and abrupt cessation of menses a year post partum. Her preceding pregnancy was uneventful, although she delivered a pair of twins. She was a non-smoker, non-alcoholic with a family history of diabetes (father had type 2 diabetes on oral hypoglycemic agents).

Immediately post partum, she noted increased thirst with a strong craving for cold drinks. She was evaluated for a suspicion of diabetes mellitus and reassured on two occasions that random blood glucose was normal. She had consulted a gynecologist for abnormal menses and was started empirically on bromocriptine for a mild hyperprolactinaemia (Prolactin = 55.7ug/L, NR 5-27.7 ug/L).

Four months later, she presented with blurring of vision, mild headaches and progressively severe polyuria (in excess of 7 liters a day), associated with poor appetite and weight loss. She was investigated by her gynaecologist and a diagnosis of new-onset type 2 diabetes was made (random glucose 26.5 mmol/L). Despite optimal glycaemic control using glibenclamide and metformin, her symptoms of polyuria and polydipsia persisted.

Endocrine testing suggested diabetes insipidus (serum osmolality of 320 mmol/kg and urine osmolality 244 mmol/kg after overnight fluid deprivation) and she was started on nasal desmopressin with symptomatic relief. Evaluation for the other anterior pituitary hormone dysfunction revealed no abnormalities (Table 1). Magnetic Resonance Imaging of the pituitary revealed a moderate thickening of the pituitary stalk (see Figure 1a).

TABLE 1 - INITIAL EVALUATION OF ANTERIOR PITUITARY FUNCTION.

Laboratory investigations	Results	Normal range
Follicle stimulating hormone (FSH)	4.8 IU/L	Follicular 1-14 Luteal 1-12
Luteinsing hormone (LH)	3.8 IU/L	Follicular 1-7.5 Luteal 2.5-24
Estradiol	179.8 pmo/L	Follicular 3.7-205.5 Luteal 176.2-1284.5
FT4	16.7 pmol/L	10.3-31
Thyroid stimulating hormone (TSH)	1.3 mU/L	0.5-5
Cortisol	373.4 nmol/L	
Growth hormone (GH)	2.5 mU/L	1.0-21.0
Insulin-like growth factor 1 (IGF-1)	152.7 UG/L	114.0-492.0

The diagnosis of lymphocytic hypophysitis was considered in view of the typical presentation. Investigations done to exclude other causes of a thickened pituitary stalk included a chest radiograph, scintigraphic bone scan and lumbar puncture. These revealed no abnormalities.

Two years after presentation, she developed secondary hypogonadism which was picked up on routine biochemical testing with FSH of 1.5 IU/L and an undetectable level of LH. Soon after, she complained of generalised lethargy and severe headaches. Investigations revealed hypocortisolism, with a random cortisol of 22.9 nmol/L and 0.25mg intramuscular synacthen stimulated cortisol of 251.2 nmol/L at 60 minutes.

Four years after presentation, she developed secondary hypothyroidism detected on blood tests. (FT4 7.3 pmol/L, TSH 0.982 mU/L)

Serial MRI scans (Fig. 1) showed evolution of the

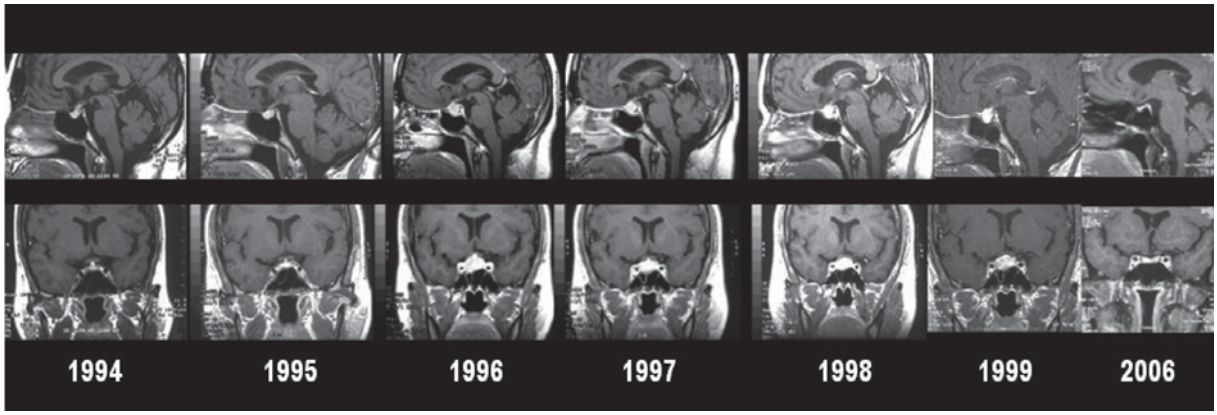

Fig. 1 - Serial MRI scans over time demonstrating a thickened pituitary stalk that evolved into a pituitary mass that progressively reduced in size leading to final disappearance of the mass.

thickened pituitary stalk to emergence of a pituitary gland enlargement giving a mass appearance, which progressively decreased in size with resultant disappearance of the mass. She remains well 14 years after initial presentation, on hormone replacement.

Discussion

This patient had first presented with polyuria and polydipsia, a year after delivery of twins. Despite control of the diagnosed diabetes mellitus her symptoms of polydipsia and polyuria had persisted; raising the possibility of another cause which was subsequently demonstrated to be diabetes insipidus. It is tempting to speculate, in retrospect, that her diabetes mellitus was aggravated by her urge to consume soft drinks in response to the polydipsia of diabetes inspidus occurring in the background of a family history of diabetes mellitus. Evaluation for the diabetes inspidus led to an identification of a pituitary stalk abnormality. Further investigations were negative for other causes of polyuria, and biochemical testing revealed normal anterior pituitary function.

A thickened pituitary stalk raises the possibilility of lymphocytic hypophysitis, as well as granulomatous or xanthomatous inflammation. Differentials include sarcoidosis, Wegener's granulomatosis, tuberculosis, Whipple's disease, Langerhans cell histiocytosis and neoplasms, including germ cell tumours, metastases or pituitary tumours.

Subsequent imaging revealed the presence of a pituitary mass. Differential diagnoses include pituitary tumours, other inflammatory conditions like sarcoidosis or histiocytosis, and malignancy. This patient underwent a bone scan and lumbar puncture, with measurement of cerebrospinal fluid beta human chorionic gonadotrophin (B-hcg) and acid fast bacilli, all of which were normal.

Serial MRI in this patient showed evolution of the thickened pituitary stalk to emergence of a pituitary gland enlargement giving a mass appearance, which progressively decreased in size with resultant disappearance of the mass.

Serial hormonal evaluation revealed progressive hormonal deficiencies finally resulting in panhypopituitarism on hormone replacement.

Because of the temporal relationship with the delivery and development of diabetes insipidus, as well as the discovery of pituitary stalk enlargement on MRI scan, the diagnosis of lymphocytic hypophysitis was considered.

The natural history of lymphocytic hypophysitis is thought to progress from inflammation to fibrosis and subsequent atrophy of the gland. Spontaneous resolution has also been reported, as illustrated in our patient. Inflammatory process of the hypophysis can be misdiagnosed as their clinical and radiological features often mimic tumours of the sellar or suprasellar region. Hyperprolactinaemia is thought to be present in a third of patients, and can be as a result of pregnancy or post partum, stalk compression or as a result the inflammatory process itself. It has been reported that lymphocytic hypophysitis should be strongly suspected if symptoms occur during or soon after pregnancy; if ACTH and/or TSH deficiency is present with normal gonadotrophin and GH secretion; and contrast enhancement scans of the pituitary gland are positive (particularly gadolinium contrast on MR scanning).

The features of hypophysitis on imaging include a homogenous enlargement of the pituitary, with a peak in the diaphragm giving it a pear shaped appearance.

Loss of the posterior pituitary bright spot and thickening of the stalk have also been reported. There are a few characteristic features on MRI scanning that can help to differentiate lymphocytic adenohypophysitis from pituitary adenomas. Adenohypophysitis have relatively low signal intensity on T1 weighted images whereas pituitary adenomas are almost isointense on both T1 and T2 weighted images. Marked contrast enhancement of the mass seems to be characteristic of lymphocytic adenohypophysitis whereas solid pituitary macroadenomas only show modest enhancement. Thirdly, dural enhancement adjacent to the enlarged pituitary seems to be typical in lymphocytic hypophysitis which is not the case in pituitary adenomas. Our patient's initial MRI showed an enlarged pituitary stalk that was enhancing brightly associated with a loss of the posterior pituitary bright spot.

There has been some suggestion that if inflammation of the pituitary gland is left untreated, the enlarged gland may shrink as the glandular tissue is destroyed by the disease process, as was the case in this patient. In many patients with lymphocytic hypophysitis, a misdiagnosis of pituitary adenoma has been made preoperatively. The distinction between a pituitary adenoma and lymphocytic hypophysitis has broad and important implications in terms of treatment, follow-up, and prognosis.

The natural history includes a progressive development of irreversible hypopituitarism or spontaneous partial/total recovery. Most patients require long term hormone replacement. Therefore, at initial presentation, the decision to sit tight and observe or to proceed with surgery is a difficult one, especially if the patient has evidence of a pituitary tumour on imaging. As spontaneous remission does occur, careful follow up of asymptomatic and subclinical patients is recommended, and to proceed with surgery if there is evidence of severe compression.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

What is the best method for assessing insulin resistance in PCOS women: comparison of static and dynamic methods and SHBG

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Introduction

Although the prevalence of insulin resistance (IR) among women with polycystic ovary syndrome (PCOS) is known to be high, the best method to use to detect IR is still controversial. The aims of this study were 1. to determine the prevalence of IR in a well-characterized population of mostly normal-weight women with PCOS, 2. to evaluate the relative agreement of both static and dynamic methods of diagnosing IR, and 3. to evaluate SHBG as a simple method for screening for IR.

Materials and methods

We analyzed data from 147 subjects diagnosed with PCOS, according to the revised Rotterdam consensus criteria, who presented to our university medical center. We collected data on clinical characteristics, fasting insulin and glucose measurements, and the results of a 3-hour, 75 g load glucose tolerance test with every 15 minute measurements of glucose and insulin. IR was defined as HOMA >3.8, QUICKI <0.33, AUC of insulin >7000 µIU*120 min/ml (AUCI120), AUC of insulin >12000 µIU*180 min/ml (AUCI180), Matsuda-Index <4.5 (ISI COMP) and Glucose to Insulin ratio <4.5 (G/I). Correlation analysis was performed between static insulin resistance indices (HOMA, QUICKI, G/I) and dynamic insulin resistance indices (ISI COMP, Stumvoll-Index (ISI STUM), area under the insulin curve over 120 and 180 minutes). We tested the diagnostic potential of SHBG as a simple screening method for those at risk of having IR. Finally, the risk for future development of DM was assessed using the formula Δ I0–30 min/ΔG0–30 x Matsuda-Index as proposed by Abdul-Ghani et al. (2007).

Results

Using our pre-defined cut-offs for IR, we found that the prevalence of IR was 12.2%, 34.0%, 60.5%, 43.5% and 55.1% using HOMA, QUICKI, AUC120, AUC180 and ISI COMP respectively. The correlation between the methods varied: HOMA and: QUICKI (r=-1, p=0.01), G/I (r=-0.958, p=0.01), AUCI120 (r=0.656, p=0.01), AUCI180 (r=0.659, p=0.01), ISI COMP (r=-0.881, p=0.01), ISI STUM (r=-0.570, p=0.01). Mean SHBG was significantly lower in women with than those without IR, when IR was defined using all methods except the QUICKI. The SHBG level strongly correlated with IR using all methods (ranging from r=-0.482, p<0.001 for AUCI120 to r=0.543, p<0.001 for ISI COMP, respectively) and was an independent predictor of the presence of IR on logistic regression. The cut-off for SHBG of ≥26.8 nmol/L had a maximal area under the curve with a specificity of 86.2% and sensitivity of 53.1% for excluding IR.
After applying a previously published formula to assess the risk of developing DM with a cut-off of <3.1, we found that 20.1% of our PCOS subjects would be deemed at risk for the future development of DM. Moreover, only the insulin resistant group categorized by AUCI180 had a significant lower ΔI0–30 min/ΔG0–30 x Matsuda index in comparison to the normo-insulinemic subjects (p=0.05).

Conclusions

We found discrepant prevalence rates (from 12.2% to 60.5%) of insulin resistance among PCOS women depending on the method and diagnostic cut-off used. A cut off of ≥26.8 nmol/L in SHBG could be used as a specific and simple screening method to exclude IR, necessitating further testing in only those who test positive. There is an evident need for standardization and agreement to use a single, most feasible method with one single cut off for IR. Most clinically important would be a method to determine IR that would be predictive of the future development of DM in PCOS women. Applying a previously published index for predicting DM in an older at-risk population to our young PCOS women, we found that over 20% would be identified as at-risk for developing DM. Whether this index proves to be a true prognosticator for the development of DM in PCOS women needs to be further evaluated in long- term longitudinal studies.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Hormone withdrawal-associated symptoms (headache and pelvic pain) in women taking combined oral contraceptives: comparison of estradiol valerate/dienogest versus ethinylestradiol/levonorgestrel

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The study aim was to show superiority of estradiol valerate/dienogest (E_2V/DNG; Qlaira®) over an ethinylestradiol/levonorgestrel (EE/LNG; Microgynon®) combined oral contraceptive (COC) in reducing the severity of hormone withdrawal-associated symptoms in women aged 18-50 y using COCs. The rationale was based on established stable E_2 levels using a shorter hormone-free interval (HFI) with E_2V/DNG vs EE/LNG. This randomized, double-blind, Phase IIIb study assessed headache/pelvic pain severity, recorded using a visual analog scale of 100mm during cycle days 22-28/6 treatment cycles. Of 449 women randomized, 362 completed the study. Study drugs were well tolerated and AEs were typical of COC use. E_2V/DNG (26/2) reduced the most severe symptom (headache or pelvic pain) significantly more ($p<0.0001$) than EE/LNG (longer HFI, 21/7). Women taking E_2V/DNG used considerably less pain medication (Fig. 1).

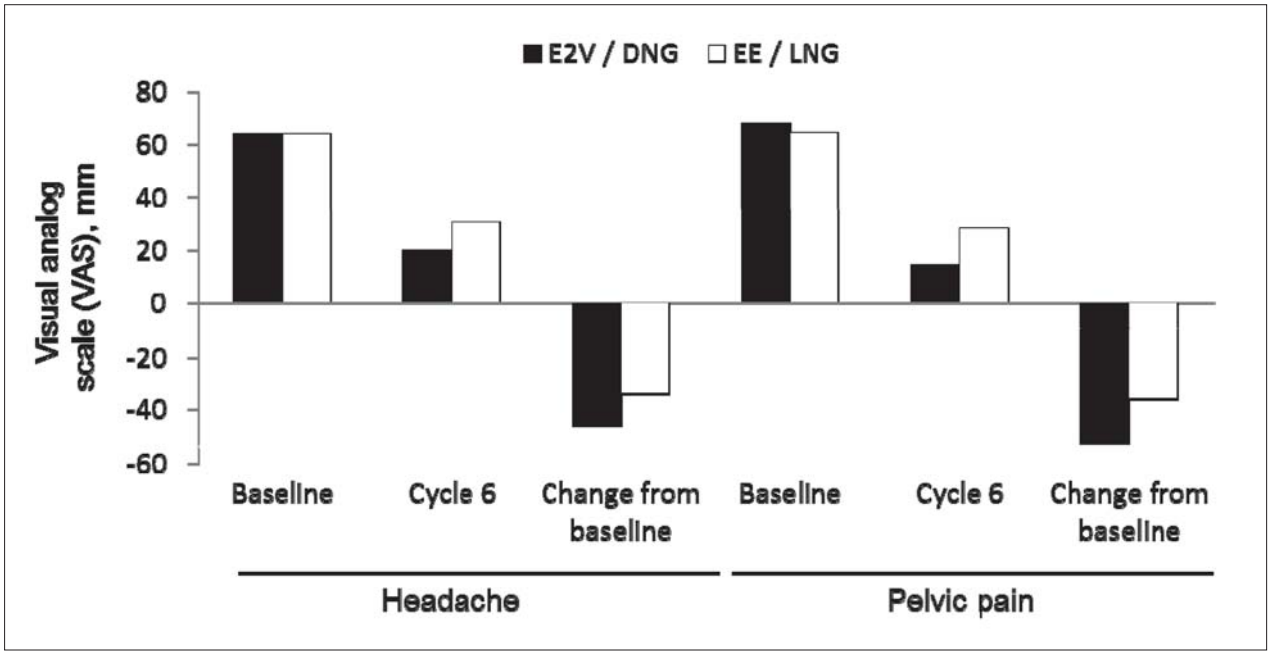

Fig. 1 - Mean Visual Analog Scale (VAS) scores as recorded by the participants - severity of headache and severity of pelvic pain.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Nuclear factor-kappa B (NF-kappa B) expression in the endometrium of the normal and pathological uterus

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Introduction

Nuclear factor Kappa.B (NF-kappa B) plays an important role in the regulation of the inflammatory cascade through the activation of several genes. The translocation of this transcriptional factor from the cytoplasm to cell nuclei involves the phosphorylation of its inhibitor, a reaction that occurs in response to several inflammatory mediators. In the endometrium, progesterone exerts an inflammatory effect that is mediated through the inhibition of the mechanism of NF-kappa B translocation to the nucleus. Progesterone withdrawal, on the other hand, leads to up-regulation of endometrial cyclooxygenase-2 (COX-2) and subsequent increased levels of prostaglandins (PGs), namely, PGE_2 and $PGF_{2\alpha}$, which provoke the onset of menstrual blood flow and regulate its intensity (1,2). Nuclear factor KappaB p65 immunoreactivity was positively associated with heavier menstrual flow in women with adenomyosis, thus suggesting a role for inflammation in the regulation of menstrual-related symptoms and uterine pathology (3). Increased levels of mRNA for Cox-2 were also observed in the endometrium of patients complaining of menorrhagia (2). These findings corroborate the hypothesis that there is an increase in the inflammation in the endometrium of patients with estrogen-dependent pathology. The present study was designed to determine whether the presence of endometrial polyps, adenomyosis, myomas and endometriosis is associated with increased expression of nuclear factor kappa B (NF-kappa B) in the endometrium during the different phases of the menstrual cycle.

Patients and methods

NF-kappa B expression was investigated in the endometrium of 69 patients of reproductive age with adenomyosis (n=40), submucous myoma (n=10), endometrial polyps (n=5) and endometriosis (n=14), and compared with 36 normal, pathology-free controls. Endometrial samples were obtained during either surgical or diagnostic hysteroscopy performed in different phases of the menstrual cycle. The presence of positive nuclear expression was determined using immunohistochemistry as previously described (4). The chi-square test was used to compare differences in percentages, with significance established at $p<0.05$.

Results

In the endometrium, NF-kappa B nuclear expression was detected mainly in the glandular epithelium and, less frequently, in the stroma (Figure 1). In pathology-

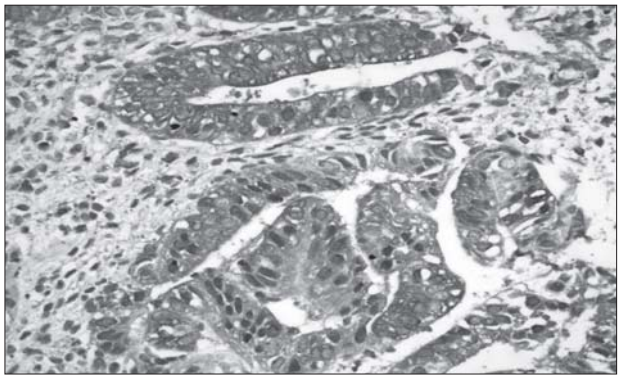

Fig. 1 - Positive nuclear NF-Kappa B expression in the endometrial glands in a patient with adenomyosis.

free patients, the percentages of endometrial samples showing positive NF-kappa B in glandular cell nuclei were low and there were no significant changes throughout the menstrual cycle. In uteri with pathology, on the other hand, the percentage of endometria with positive NF-kappa B expression was 68% in the proliferative phase, 17% in the early luteal phase and 87% in the late luteal phase. Compared to controls, these values were significantly higher ($p<0.05$) in each phase of the cycle except for the early luteal phase ($p=0.2$). These results are summarized in Table I.

TABLE 1 - PERCENTAGE OF ENDOMETRIAL SAMPLES SHOWING POSITIVE NUCLEAR NF-KAPPA B EXPRESSION IN THE GLANDS DURING THE MENSTRUAL CYCLE IN RELATION TO THE PRESENCE OR ABSENCE OF UTERINE PATHOLOGY.

	Proliferative phase		Early luteal phase		Late luteal phase	
Normal uterus	6/21	28%	0/6	0%	1/9	11%
Pathology	19/28	67%	3/17	18%	21/24	87%

Chi square test
p<0.0001 Pathology, early luteal phase versus late luteal phase
p=0.001 Pathology, early luteal phase versus proliferative phase
p=0.09 (NS) Pathology, late luteal phase versus proliferative phase

Differences between pathology and the normal uterus were significant ($p<0.05$) except for the early luteal phase.

Conclusion

The presence of pathology is associated with increased NF-kappa B expression in the endometrium and this is hormonally regulated. Progesterone decreases while estrogen and progesterone withdrawal exert an opposite effect. The increased positivity for nuclear NF-Kappa B during the proliferative and late luteal phase in the endometrium of uteri with pathology suggests that both estrogenic dominance and progesterone withdrawal increases inflammation. The reduction in the early luteal phase is in accordance with the concept that progesterone exerts an anti-inflammatory effect on the endometrium through the NF-Kappa B pathway (1,5). Estrogens potentiate TNF alpha activation of NF-Kappa B in endometrial cells, while progestins such as dienogest or gestodene exert a converse effect, blocking the translocation of NF-kappa B to cell nuclei, which otherwise would activate the transcription of pro-inflammatory genes (4,5). TNF is a potent inducer of Cox-2 in the endometrium, acting through NF-Kappa B. However the degree of induction was significantly greater in the eutopic endometrial stromal cells of patients with endometriosis compared to disease-free cases (6). This would suggest a greater activation of the NF-Kappa B pathway in the eutopic endometrium of women with endometriosis, which is in agreement with our findings of greater positivity for nuclear NF-Kappa.B in the endometrial cells of uteri with adenomyosis, myomas and endometriosis. Our findings that the binding of NF-Kappa B to the cell nuclei of endometrial cells is greater during the late rather than the early luteal phase may be a consequence of estrogen and progesterone withdrawal following the demise of the corpus luteum. This stimulates COX-2 expression and PGF2 alpha production through NF-kappa B activation, which may be responsible for the excess menstrual bleeding and pain associated with these pathologies. The role of inflammatory mediators such as prostaglandins in the menstrual process is pivotal in determining the intensity of its related symptoms, which have an endometrial origin. Excessive endometrial inflammation may either trigger or be a consequence of the aberrant aromatase expression in the endometrium, thus creating a vicious cycle of excessive local estrogen and prostaglandin production (9). The increased nuclear binding of NF-Kappa observed during the phases of menstrual cycle in which there is either estrogenic dominance or progesterone withdrawal may suggest that these steroid hormones exert conflicting effects in the regulation of endometrial inflammation.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Effects of different bariatric surgery procedures on plasma total antioxidant capacity in women with severe obesity

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Introduction

Insulin resistance (IR) in obesity is related to the development of metabolic syndrome (MS) and pituitary-ovarian axis disorders (1). Severe obesity, after failure of dietetic and pharmacological treatment, can undergo bariatric surgery to prevent morbidity and mortality (2). Oxidative stress (OS) is now recognized as a mechanism underlying the development of cardiovascular complication of MS. The evidence that obesity is related to an increase in OS has been highlighted (3-6), but few studies have comprehensively examined antioxidant systems in human obesity. In previous works we demonstrated that weight loss, induced by biliopancreatic diversion (BPD), is associated with increased insulin sensitivity, but decrease of molecules, such as Coenzyme Q_{10}, that act as powerful antioxidant system, with mechanisms (weight loss *per se*, lipid malabsorption, metabolic or hormonal variations) which are not yet clear (7). In order to delve into the physiopathology of this condition, we have studied three different groups of subjects, treated by different surgical procedures - BPD, gastric bypass (GB), mini-gastric bypass (GmB) - evaluating total antioxidants capacity (TAC) in plasma before and at a long distance (12-18 months) from surgery.

Materials and methods

We studied 14 women, aged 29-48 ys, with a mean BMI ± SD of 47.5 ± 6.8 Kg/m^2; no other endocrine disorder was present except amenorrhea or oligomenorrhea; after surgery they reached a mean % decrease of BW of 40.7 ± 0.1, with no differences among the techniques, and with normalization of menses. They were treated with the same schedule of supplements. We studied metabolic parameters (glycemia, insulinemia, total- LDL- HDL-cholesterol, triglycerides, uric acid, albumin, transaminases), hormones (FT3, FT4, TSH, IGF1, cortisol, ACTH) and values of copper, zinc and Vitamin E. IR was evaluated by homesostasis model assessment (HOMA), calculated as basal glucose (mmol/l) x basal insulin (mIU/l)/22.5, where high HOMA scores ($\geq$2.5) denote IR (8). Total antioxidant capacity (TAC) was assayed by a method based on interaction between H_2O_2-metmyoglobin, as source of radicals, and a chromogen (ABTS), whose radical cation is spectroscopically revealed The latency time (LAG, sec) in $ABTS^{\cdot+}$ appearance is proportional to antioxidants concentration (9,10).
Mann-Whitney test was employed for statistical evaluation.

Results

Despite in BPD different parameters were markedly influenced by the procedure, in comparison to the other techniques (Table 1), due to the prevalent

TABLE 1 - MEAN ± SEM VALUES OF METABOLIC PARAMETERS AND OLIGOELEMENTS IN THE THREE GROUPS OF BARIATRIC SURGERY TECHNIQUES.

	HOMA	HDL-Chol mg/dl	IGF-1 ng/ml	Zn γ/dl	Cu γ/dl	Vitamin E mg/l
BPD	1.5 ± 0.3	38.8 ± 2.3	51.4 ± 34.3	67.7 ± 26.4	104.5 ± 6.4	8.1 ± 4.3
GB	1.4 ± 0.6	51.3 ± 6.4	170.0 ± 53.5	90.0 ± 16.2	105.8 ± 25.4	10.9 ± 2.9
GmB	0.5 ± 0.3	61.8 ± 6.7	73.0 ± 47.6	82.5 ± 24.4	92.6 ± 18.3	11.0 ± 1.3
p	*0.002*	*< 0.001*	*0.001*	*0.321*	*0.519*	*0.249*

lipid malabsorption mechanism, we found a decrease in TAC in all three groups (mean % decrease of LAG values: 12.2 after BPD, 18.4 after GB, 13.0 after GmB) (Fig. 1).

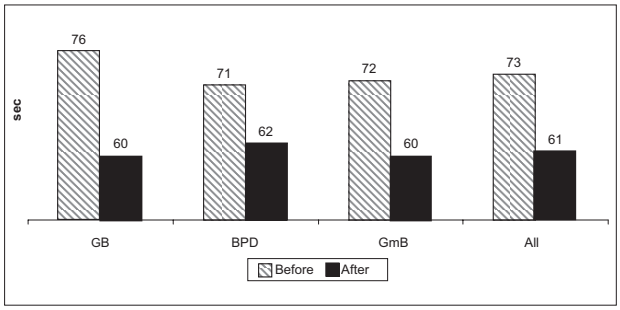

Fig. 1 - Mean values of TAC, expressed as lag phase, in the three groups of bariatric surgery techniques.

Other parameters did not show significant differences among groups (data not shown).

Discussion

BPD is a surgical procedure performed in patients with untreatable obesity and insulin resistance and consists in partial gastrectomy with Roux-en-Y reconstruction (2). As a consequence, nutrition does not undergo the normal action of biliary and pancreatic secretions in alimentary tract; the patients develop fat malabsorption and a partial starch malabsorption, while maintaining absorption of mono-disaccharides and proteins (11). Among the consequence of lipid malabsorption we evidenciated a marked reduction in Coenzyme Q10 (7). Different techniques have been developed to reduce the impact of such mechanism (12).

A large group of studies has been published on the role of OS in the development of MS. In the context of a genetic predisposition to IR, induced by excessive caloric intake or sedentary lifestyle, it has been hypothesized a diet-induced OS (13); the chronic free fatty acids overflow can worsen insulin sensitivity (the so colled "lipotoxicity") inducing a reduction in insulin-dependent glucose uptake (14). Insulin receptor substrate phosphorylation is involved in OS-induced IR (15). The evaluation of TAC fournishes an indirect evaluation of OS, which is defined as an unbalance between the production of radicals and the defence systems. We also observed TAC values in morbid obesity not significantly different from those in control subjects (16).

Our present preliminary data show a reduction in TAC, measured as lag phase, after all the techniques of bariatric surgery, suggesting that body weight reduction per se, rather than the different surgical procedures, can be responsible for this phenomenon.

This datum, which could indicate an initial decrease in OS (and therefore a reduction of the compensative antioxidant activity), could appear as a favourable index; however, at a longer distance, it could be a mechanism contributing to the well known phenomenon of weight regain (17).

We suggest that antioxidants should be determined in patients undergoing bariatric surgery, despite this is not a routinary measure as recommended (18) and, in case of persistent reduction, supplemented according to a personalized approach. However, further study can gain insight into the differential patterns of specific antioxidants, which can contribute to the antioxidant power in serum.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Definition of National norms of quality of life, and manifestation of climacteric syndrome in Armenian women population in 40-60 age group

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Aim

Definition of National norms of quality of life, and manifestation of climacteric syndrome (CS) in Armenian women population in age group 40-60 years.

Objectives

1. To present a comparative assessment of the menopausal syndrome in women population of the main cities of Armenia.
2. To assess the parameters of physical and mental health of women of the capital and major cities of Armenia.

Materials and methods

The study is based on data cross-sectional epidemiological study (survey) of 900 women aged 40-60 years in four major cities of Armenia:

- Yerevan, the capital of the Republic;
- Gyumri and Vanadzor, the victims of the earthquake in 1988;
- Stepanakert, participated in local armed conflicts.

The sampling method of partial observation was used, which allowed to extend the results from part of the units (we have chosen the group) to the whole population. To ensure the representativeness of our sample the necessary requirements were fulfilled according to calculation of sample size and selection of observation units in the sample.

The study of the quality of life (QOL), as well as evaluation of physical and mental health components were carried out using SF-36 (1-3). SF-36 questionnaire, the official Armenian valid version which is developed by specialists at the American University in 2002 (G. Yagdzhyan, D. Abrahamian, A. Vanesyan in the Center for Plastic Surgery and Medical Science of Armenia) is a widely used general questionnaire that measures QOL related to health and its eight areas (subscales), i.e.

1. Physical functioning - PF (10 points)
2. Role limitations due to physical health problems – RP (4 points)
3. Bodily pain - BP (2 points)
4. General health - GH (6 points)
5. Vitality - VT (4 points)
6. Social functioning - SF (2 points)
7. Role limitations due to problems in emotional functioning - RE (3 points)
8. Mental Health - MH (5 points).

The most pronounced is manifestation of the CS in Gyumri and Stepanakert, where menopausal index corresponds to the severe form of the CS. The population of the age group 40-60 years in Yerevan and Gyumri are characterized by medium degree of expression of CS.

1. Physical functioning (PF) – represents the activities that normally performed daily. The health status limitation is the lowest in women population of Yerevan compared to other cities. The highest limitation determined in Gyumri. In half of population of Yerevan, Vanadzor and Stepanakert the physical functioning is above average as opposed to Gyumri, where (PF) is estimated as below average.

2. Role limitations due to problems in physical health (RP) – characterized by difficulties in work or other regular daily activities due to physical condition. Problems with work and daily activities due to physical health dis-

orders occurred in the least degree in women population of Yerevan and quite often in women population of Gyumri and Vanadzor. It is interesting to mention that 50% of the population of Yerevan and 40% of the population of Stepanakert noted that there are no problems connected to work and other daily activities due to physical health, while one of three women in Gyumri and Vanadzor, revealed significant difficulties.

3. Bodily pain (BP) – the severity of physical pain experienced in the last 4 weeks, as well as its influence on the performance of daily work (outside and housework). The lowest average score in a comparative aspect on the bodily pain scale scored women population of Gyumri, moreover, for the past 4 weeks pain significantly interfered in normal daily life of women population of this city, as the points scored by half of women (50th percentile = 46) was below average (51.3).

4. General health (GH) – the overall self-appraisal of health and its dynamics for the last year, where the women describe their health as a whole, comparing their health at the time of the survey, to the fact that was one year ago. Calculations have shown that the highest self-appraisal of health is in women population who live in the capital. Below all rated their health women of Stepanakert, although more than in half of the studied women (75%) the indicator of general health is twice the average.

5. Vitality (VT) – representation about the safety of vital forces concerning to how respondents felt during the last four weeks. Relatively more state exhaustion and fatigue accompanied by women living in Gyumri. Average values on a scale of VT in three other cities approximately the mid-level, which indicates that women of these research groups basically gave answers about a feeling of cheerfulness, fullness of energy.

6. Social functioning (SF) includes the question of how physical or emotional condition within the previous month disturbed to spend time with family, friends, neighbors, or in a team. On a scale SF women of the capital gained (68.8), the half the population (median) exceeds the level of social activity (75). In the cities Vanadzor and Stepanakert the average values are almost close to the capital (67.1 and 66.6, respectively), but the level of social activity of every second woman of mentioned cities remains below average (62.5 and 62.4, respectively). Comparative evaluation showed that for the last 4 weeks women living in Gyumri compared to women living in the rest of mentioned cities, had more problems with physical health and emotional disorders interfering with various habitual communication and social life (meetings and visits to friends, relatives and etc.).

7. Role limitations due to problems in the sphere of emotional health (RE) – if there were emotional difficulties at work and routine activity during the last 4 weeks. Comparative analysis of the calculations showed that most difficulties because of emotional instability, experienced women of Gyumri. The level of the average value on the scale RE in Gyumri is almost 2 times lower than in Yerevan and Stepanakert. The status of "floor" and "ceiling" are clearly displayed on the scale: 47% of women of the capital and 42% of women of Stepanakert scored the highest possible score, indicating that there are no issues with work and daily activities caused by emotional state. Totally different is the situation in 2 other cities: 44% of women of Gyumri and 46% of women of Vanadzor were forced to reduce to minimum the amount of working time and volume of work due to serious emotional disorders.

8. Mental health (MH) – the mood of respondent during the last 4 weeks. The highest average scores for all parameters obtained by women in the capital - (54.2). Feeling of nervousness, depression, grief and discouragement are relatively often accompanied by women of Gyumri (46.5), thus, the emotional state with a tendency to depression made the women of this city psychologically vulnerable.

Conclusion

For the first time were obtained the national norms of quality of life necessary to carry out scientific researches in the given age group of women. The quantitative indicators of received norms in comparison to similar data of developed countries reveal a number of anomalies, the explanation of which requires further investigation. The lowest normative indicators for all eight parameters of the questionnaire SF 36, obtained in Gyumri, located at approximately the same level as the norms from the United States. What could this mean? High self-appraisal? Underestimated requirements of quality of life? Or higher mental and physical stamina? The answers to these questions require further research.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Comparison of various progestins in the preoperative treatment of endometriosic cysts

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Introduction

Endometriosis is a common disease defined as the growth of endometrial tissue outside the uterine cavity and results in a vast array of gynecological problems including dyspareunia, dysmenorrhea, pelvic pain and infertility.Progesterone is a potent antagonist of estrogen?induced proliferation in the endometrium and may play a pivotal role in the pathogenesis of endometriosis. There is some evidence to justify using hormonal drug treatments following surgery to suppress the growth and development of any remaining or new endometrial implants.The progestins are effective treatments for the symptoms of endometriosis. However, like all the hormonal drugs used for endometriosis, they have side effects, which some women find intolerable. They are safer and cheaper than the GnRH-agonists and danazol, which some gynaecologists believe makes them appropriate for women who need prolonged or repeated treatments. It is not known precisely how progestins relieve the symptoms of endometriosis, but they probably work by suppressing the growth of endometrial implants in some way, causing them to gradually waste away. They may also reduce endometriosis-induced inflammation in the pelvic cavity. Unfortunately, there is no consensus in the literature on the optimal dose and treatment time for lessions'regression. Furthermore, there are very few data on the type of progestin that is most effective, and the regimens advocated are essentially arbitrary. Our primary goal was to assess the differences between the results of various progestins regarding amelioration of clinical symptoms, ultrasonographic findings and intraoperative bleeding, adhesions and ease of cyst dissection.

Patients and methods

We performed a retrospective single-center study including one hundred fifty patients diagnosed with endometriosic ovarian cysts operated in the University Women's Hospital Cuza Voda Iasi, Romania, in the period 1.06.2005- 01.06.2010 which followed at least 6 months of preoperative treatment with various progestins. The diagnostic circumstances were pelvic examination or vaginal ultrasound performed to evaluate pelvic pain (*n* = 68), ovarian stimulation *(n* = 14), initial infertility workup (n = 39), or routine gynaecological examination (*n* = 29). All cysts were > 20 mm in diameter, and none had thick septa, papillations, or associated ascites. All patients were examined by vaginal ultrasound with associated Doppler at the moment of diagnosis and every three months after and met the classical ultrasonographic criteria for endometriosic cysts. We assessed the cysts dimensions, appearance, shape, vascularisation, situation related to the uterus and the controlateral ovary, uni- or bilaterality of the cysts, the vascularisation, sludging and presence of horizontal levels. The diagnosis was confirmed in all cases postoperatively by histological examination, which demonstrated the presence of histopathologic features similar to endometrium, namely endometrial stroma, endometrial epithelium, glands that respond to hormonal stimuli and haemosiderin deposits. 33 (27%) patients followed treatment with lynestrenol (Exluton), 23 (23%) were prescribed injectable medroxyprogesterone acetate (DepoProvera), and 76 (50%) were treated with desogestrel (Cerazette). Age of the patients varied between 20-49 years (with an average of 32), all of them being still menstruating. 53 patients were referred for associated infertility. Dura-

tion of treatment was 6-12 months before laparoscopy and 3 other months after surgery. The parameters followed intraoperatively were the ease of dissection, the amount of bleeding, the possibility of conservative treatment, the extension of adhesions, the staging of disease. The bleeding was subjectively assessed as mild, moderate and severe. Postoperatively the patients were reevaluated every three months by clinical examination and vaginal ultrasound.

Results

The characteristics of the three groups were compared using Student's *t*- and χ^2-tests. A significant decrease of cysts diameter and vascularisation was found in most cases, usually associated with sludging and decreased hyperechogenicity, as shown in Table 1. The most evident improvement of the ultrasonographic appearance regarding size decrease and sludge formation belonged to patients treated with desorgestrel. Vascularisation decrease and horizontal levels appearance were similar for all progestins.

TABLE 1 - ULTRASONOGRAPHIC PREOPERATIVE CHANGES.

	Lynestrenol	Medroxyprogesterone	Desorgestrel
Size decrease (mm)	29	31	38
Vascularisation decrease (%)	79	82	85
Sludge appearance (%)	27	25	37
Horizontal levels appearance (%)	31	28	32

TABLE 2 - SURGICAL PARAMETER MODIFICATIONS FOR DIFFERENT PROGESTINS.

	Lynestrenol	Medroxyprogesterone	Desorgestrel
Easy dissection and capsula cleavage (%)	80	85	89
Heavy bleeding (%)	12	10	5
Thick adhesions (%)	34	25	21
Conservative treatment (%)	98	95	100
Operative time (min)	85	90	79

TABLE 3 - POSTOPERATIVE FOLLOW-UP.

	Lynestrenol	Medroxyprogesterone	Desorgestrel
Recurrence (%)	6	7	5
Pregnancy in the first 6 months after progestin interruption (%)	79	82	85
Symptom-free (%)	87	89	92

Intraoperatively we noticed less bleeding during cyst dissection and a better cleavage plan between the cyst capsula and the normal ovarian tissue (Table 2). These effects were most visible in the desorgestrel group. 55% of the patients obtained a pregnancy in the first year after laparoscopy and another 12% during the second year. The overall recurrence rate of endometriosic cysts after stopping the progestative treatment was 6% (Table 3).

Discussion

This study showed that treatment with progestative improves operative conditions mainly by decreasing the bleeding and increasing the laxity of the connective tissue between the cyst capsula and the normal ovarian tissue. Since the main effect of progesterone on endometrium is decidualisation of stromal cells, these changes could be due to a local decrease of angiogenesis and increase of glycan cell content. There has been some controversy regarding the distribution of progesterone receptors A and B in endometriosis, which have been reported to be both low and unusually high in endometriotic tissue. The slightly different effects noticed in various progestatives could be owed to some differences in their affinities for the two types of receptors. There was no case of complete resistance to the treatment in any group. We are not aware of another study comparing the effects of different progestins in endometriosis, but there is a recent study which does not find a significant difference between the effects of various progestins in endometrial hyperplasia.

Conclusions

Desorgestrel, lynestrenol and medroxyprogesterone administered for at least 6 months preoperatively have a shrinking effect on endometriosic cyst, decrease the intraoperative bleeding and improve the dissection conditions, with desorgestrel having the most significant effects. Further studies at molecular level and more extended clinical trials are necessary to standardise the progestative preoperatory treatment in endometriosis.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Desorgestrel prevention of recurrences after surgical treatment of ovarian endometriosic cysts

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Introduction

Endometriosis is a relatively frequent chronic gynecologic disease usually presenting with infertility or chronic pelvic pain. The confirmation of the disease can only be made with surgical intervention, usually laparoscopy. Endometriomas are particularly difficult to excise because surgical intervention may reduce ovarian reserve, the capsula is very adherent and well vascularised. The surgical treatment of endometriosis is effective in the short-term, but if the patient does not get pregnant very soon after the operation, the recurrence is common. Recurrence rates after surgical intervention can be decreased with the use of menstruation suppressive medical therapy such as hormonal contraceptives.
Desogestrel is a progestogenic 19-nortestoterone derivative, which is widely used for oral contraception both combined with ethinyl estradiol and alone in progestin-only pills. The parent drug does not possess progestogenic activity and bioactivation to 3-ketodesogestrel is needed for the contraceptive activity. In one parralel study we demonstrated it has properties superior to other progestins in the preoperative treatment of endometriotic lesions, therefore we studied its effects when the treatment was continuated postoperatively in patients operated for endometriosic cysts.

Aim of the study
To evaluate the effects of a continuous desorgestrel treatment in the prevention of ovarian endometriosic cysts postoperative recurrence.

Materials and methods

Our study design was retrospective, including 120 patients with endometriosic cysts operated in the University Women's Hospital Cuza Voda Iasi, Romania during the period 1.06.2005-1.06.2010. The cysts were diagnosed during pelvic examination or vaginal ultrasound done for infertility, chronic pelvic pain, abnormal vaginal bleeding or routinely. The minimum cyst diameter was 20 mm and patients which had cyst septa, papillae or peritoneal fluid were excluded from the study. The patients followed at least 6 months of preoperative treatment with various progestins. 105 patients were operated laparoscopically and 15 had a laparotomy, from which 11 were converted laparoscopies and 4 first intention laparotomies due to the big size of the cysts and/or previous extended abdominal surgery.
We evaluated the cysts size, homogenicity, shape, vascularisation, position related to the uterus and the controlateral ovary, presence of sludging and horizontal levels. All cases were confirmed by pathology examination showing the histological aspects characteristic to endometriosis: endometrial stroma, endometrial epithelium, glands that respond to hormonal stimuli and haemosiderin deposits. 80 patients received for at least 6 months 75 micrograms of desorgestrel daily and 40 patients did not receive any treatment. Vaginal ultrasound with Doppler examination and CA 125 were done for follow-up at every 3 months. The characteristics of the two groups were compared using Student's *t*- and χ^2-tests.
Age of the patients varied between 18-47 years (with an average of 34), all of them being still menstruating. 53 patients were referred for associated infertili-

ty. 37 out of the 40 patients who did not receive any postoperative treatment were infertile and willing to be able to conceive as soon as possible after the operation.

Results

Desorgestrel was well tolerated and no patient had to withdraw because of side effects. 48 women became amenorrhoeic, 23 had irregular vaginal bleeding and 9 women had regular periods. The bleeding amount was described as much lower than a normal period. 12 patients complained of weight gain, the maximum being 5 kg. 5 patients complained of vaginal dryness and sexual discomfort. 4 patients treated with desorgestrel had another laparoscopy after 6 months to check tubal patency and in none of them recurrent lesions were seen.
The cyst recurrence rate was significantly lower (5%) in the desorgestrel group than in the control group (37,5%), as well as the pelvic pain, dyspareunia and dysmennorrhea. The main side effects during desorgestrel treatment were amenorrhea, irregular bleeding, weight gain and decreased libido.

TABLE I - RECURRENCE RATE OF CYSTS AND SYMPTOMS IN THE STUDY GROUPS.

	Desorgestrel group (n=80)	No treatment group (n=40)
Cyst recurrence rate at 6 months, %	5	37,5
Pelvic pain	6	15

Conclusions

According to the Practice Committee of the American Society for Reproductive Medicine (2008), 'endometriosis should be viewed as a chronic disease that requires a life-long management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures'. Medical treatment should reach two main goals: relief of pain for prolonged periods and prevention of disease progression during the interval between conservative surgery and conception seeking.
Continuous desorgestrel treatment significantly prevents recurrence of endometriosic cysts and clinical symptoms related to endometriosis. Desorgestrel offers the associated benefits of a well-tolerated contraceptive and allows a safe delay for child planning without risking a recidive of the disease. This therapy is low-cost, efficient and brings a considerable improvement of patients' quality of life.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Treatment of endometriosis with local injection of aspirin. Experimental study in rabbits

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Introduction

Endometriosis is a chronic condition characterized by the presence of endometrial tissue outside the uterine cavity. It affects between 2 and 7% of young women of mainly of reproductive age (1-3). The first and best treatment option is surgery, even so, recurrence rates are high at 47% (4-6). Recent treatment studies have aimed to reduce the number of invasive procedures, improve prognostic evolution, and reduce recurrence indices.
However, testing treatments with new medications requires experimental models prior to clinical tests with humans. We decided to use acetylsalicylic acid (aspirin) in this study because of its widespread use and previous experimental data that we have gathered on the subject (9-14).

Materials and methods

Animal housing and endometriosis induction
Forty female adult virgin rabbits were used, weighing approximately 1500g; All animals were submitted to implant of endometrium according to technique described by Silva *et al*. (2004) (17).

Second surgical procedure and groups
Thirty days after endometrial tissue implants, all animals were randomly allocated into four groups (10 rabbits per group): Group 1 (control): treated with physiological solution; Group 2 (acetylsalicylic acid): treated with acetylsalicylic acid; Group 3 (Control): treated with physiological solution; and Group 4 (acetylsalicylic acid): treated with acetylsalicylic acid.

Solution test
A 5% solution was obtained by diluting 2500mg of acetylsalicylic acid in 50ml 10% sodium bicarbonate and applied by insulin syringe and needle directly into the endometriosis foci.

Protocols 1 and 2
The early effects were evaluated by sacrificing rabbits (Groups 1 and 2) 24h after (Protocol 1), and the late effects by sacrificing rabbits (Groups 3 and 4) 10 days after the end of treatment (Protocol 2).

Third surgical procedure
After sacrifice, all rabbits were submitted to a third laparotomy to collect and perform gross analysis on specimens. All gross characteristics and sizes (width and length) of all endometriotic implants were evaluated using a pachymeter.

Effects of treatment evaluation
Fragments were prepared for mounting on histological slides. After histological evaluation, the whole remaining area of endometrial tissue was analysed using OPTIMAS® 6.1 System imaging software.

Statistical analysis
To analyze the results Kolmogorov-Smirnov, ANOVA and Tukey test was used.

Results

Protocol 1

Group 1–Control
Histopathological analyses of these 9 lesions were consistent with endometriosis (Fig. 1).

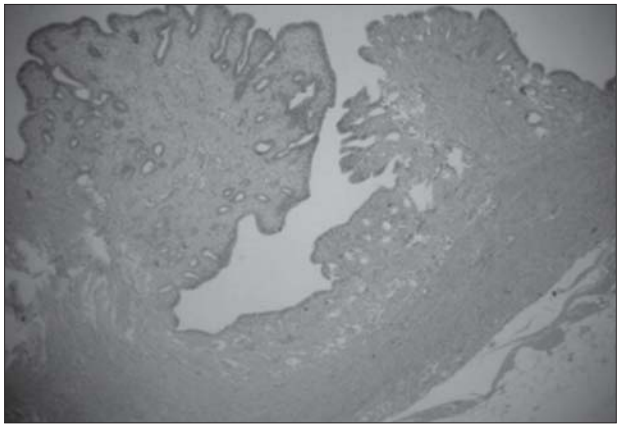

Fig. 1 - Group 1 (Control) after 24 Hour – Well developed cystic formation with bleeding foci, visualized in stromal vessels. (10x – HE staining).

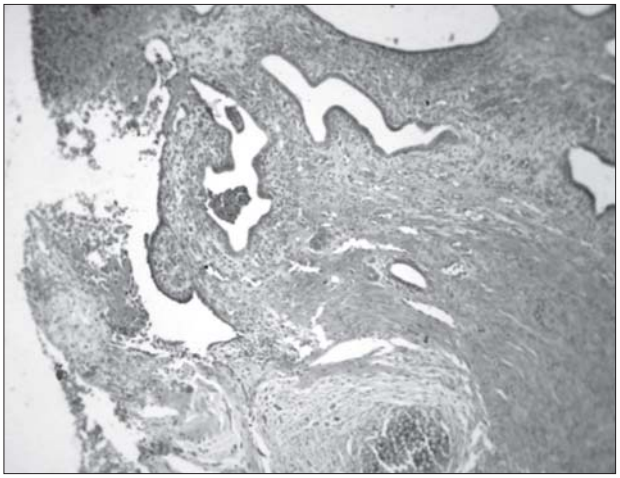

Fig. 2 - Group 2 (Acetylsalicylic acid) after 24 Hour – Granulomatous reaction around the suture thread formed by newly formed conjunctive tissue and covered by tall columnar epithelium of fusiform cells. Differently to no treated tissue (PBS) sinuous endometrial glands and hemo-neutrophilic exudate (arrows) can be seen. (10x magnification – HE staining).

Group 2–Acetylsalicylic acid

Microscopically, all collagen capsule neoformed cyst slides were covered by a fusiform columnar epithelium, associated with neutrophil exudation differing from the endometriosis cysts on the Control group slides (Fig. 2).

Control Group versus Acetylsalicylic acid Group

A significant difference was found between the two groups. The affected area in acetylsalicylic acid- treated animal implants was smaller than in the control group one day after treatment; this was also observed in analysis of the remaining endometrial tissue.

Protocol 2

Group 3–Control

Microscopic analyses were consistent with endometrial tissue cyst (Fig. 3). The endometrium was in the proliferative phase, demonstrating that the endometrial tissue was in free growth during this period.

Group 4–Acetylsalicylic acid

Histopathological evaluation of these lesions revealed endometrial tissue with rare stroma in 7 implants, only one of these with a bleeding focus. On 6 slides, endometriotic foci with their respective stroma were surrounded by weak conjunctive tissue delimiting endometrial islands. Inflammatory cells could be seen in the formed lumen, thus maintaining columnar epithelial integrity (Fig. 4).

Control Group versus Acetylsalicylic acid Group

A significant difference was found between the two groups. The affected area in acetylsalicylic acid- treat-

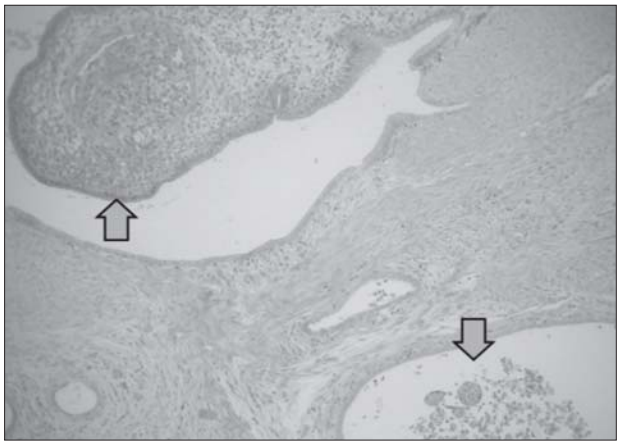

Fig. 3 - Group 1 (Control) after 10 days - Endometrial implant and cystic configuration, covered by epithelium in proliferation. (10x magnification – HE staining).

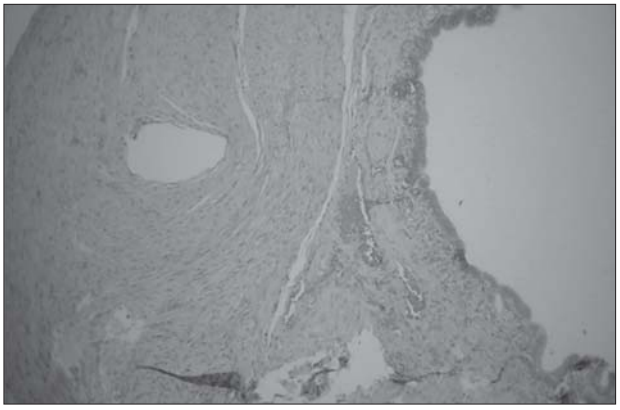

Fig. 4 - Group 2 (Acetylsalicylic acid) after 10 days - Clusters of endometrial tissue isolated by conjunctive tissue, circular formations of endometrium, showing inflammatory exudate from stroma composed of neutrophils, macrophages with intracellular lipid content, and cell debris (arrows) (10x magnification – HE staining).

ed animal implants was smaller than in the control group 10 days after treatment; this was also observed in analysis of the remaining endometrial tissue.

Gross (area) and histological (percentage remaining) measurements
Tables 1 and 2 show that the percentage of remaining viable endometrial tissue was much lower in acetylsalicylic acid-treated groups 2 and 4 than that in saline solution groups 1 and 3 ($p<0.0001$); this was also true for total lesion area (gross evaluation).

Protocol 1 versus Protocol 2
Statistical analysis comparing protocol 1 to 2 showed no differences between the controls groups, or the acetylsalicylic acid groups ($p=0.2905$), and no differences between times ($p=0.7475$).

Discussion

Acetylsalicylic acid is a well-known inhibitor of cyclooxygenase activity, even 100 years after its discovery new possibilities are still being explored.
Based on previous reports (10,11), we decided to evaluate the effects of acetylsalicylic acid at two different moments (24 hours and 10 days after intra-lesion application); acetylsalicylic acid concentration (5%) and quantity injected in each animal in this study were also based on these studied (10-12).
The study solution effectively induced a neutrophilic inflammatory response in the first 24 hours after application. In the long term (2-10 day protocol), there was less tissue growth, which was held inside weak new conjunctive tissue. The inflammatory reaction also lasted over 10 days, but without direct destruction of epithelial tissue from the endometrium, in contrast to reports from *in vitro* studies (9,12).
The effect of acetylsalicylic acid in promoting endometrial tissue destruction was clearly observed both macroscopically by the evidence of smaller lesions and by the important decrease in viable endometrium in these lesions.
When the effect of time was analysed, no difference was seen between 24 hours and 10 days, which means that acetylsalicylic acid can destroy endometrial tissue 24 hours after injection, and that the effect continues to prevent endometrial growth over time (10 days).
We observed that physiological solution allows fragment evolution and free development of implanted tissue over time without causing any inflammatory changes.
As pointed out in the Introduction, data is scarce in literature on the use of topical and local medicines in endometriosis treatment, which makes comparing our results with other studies difficult. However, the good results found in our previous investigations (9-13) using acetylsalicylic acid and its derivatives reinforce the results shown in this study.
In this experimental study, acetylsalicylic acid directly injected into endometriosis foci was very effective in their destruction; this presents new perspectives for endometriosis treatment and its clinical application based on further clinical studies.

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TABLE 1 - MEAN AND STANDARD DEVIATION VALUES FOR ENDOMETRIAL TISSUE AREA FOR EACH TIME AND GROUP.

Time	Group	No. of observations	Mean	Standard deviation	P-value
24h	Control	10	7124.40 (A)	665.21	<0.0001
	Aspirin	10	4293.20 (B)	784.42	
10d	Control	10	7015.70 (A)	1316.18	<0.0001
	Aspirin	10	4010.40 (B)	1037.85	

Means followed by same letter do not differ significantly at 5% of probability by Tukey test.

TABLE 2 - MEAN AND STANDARD DEVIATION VALUES FOR ENDOMETRIAL TISSUE PERCENTAGE FOR EACH TIME AND GROUP.

Time	Group	No. of observations	Mean	Standard deviation	P-value
24h	Control	10	80.20 (A)	6.41	<0.0001
	Aspirin	10	10.20 (B)	4.13	
10d	Control	10	81.70 (A)	9.71	<0.0001
	Aspirin	10	7.40 (B)	2.88	

Means followed by same letter do not differ significantly at 5% of probability by Tukey test.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Endometriosis and dietary intolerance – a connection

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Introduction

Endometriosis is a gynaecological condition characterized by ectopic endometrial tissues located outside of the uterus, most commonly found on the pelvic peritoneum or ovary. Endometriosis, occurs in about 7-10% of women in the general population and 71-87% of women with chronic pelvic pain (Ozawa et al. 2008). The pathogenesis of this gynaecological condition remains enigmatic and its long-term management may be inadequate in a substantial number of patients (Szamatowicz 2008). Endometriosis may be totally asymptomatic or reveal itself by severe intractable dysmenorrhoea, menorrhagia, and dysparuenia. Distressing complications related to endometriosis also include infertility and gynaecological surgical interventions for endometriomas and adhesion formation. In some cases the only definitive treatment is a total abdominal hysterectomy and bilateral salpingo-ophorectomy.
The anatomical proximity of the female reproductive organs and the gastrointestinal tract may infer at the possibility of a relationship between both systems. Endometriosis has been shown to be a significant cause of gastroenterologic distress in young women. Clinical manifestations vary considerably depending upon the anatomic extent of disease, abdominal complaints and physical findings frequently resulting in misdiagnosis and delayed recognition of gastrointestinal involvement (Zwas et al. 2008). Moreover endometriosis may coexist with or be misdiagnosed as inflammatory bowel disease (Seaman et al. 2008).
Gastrointestinal symptoms may suggest bowel pathology or possible dietary intolerance. The latter possibility is related to immunological surveillance which may be altered even in the peritoneal milieu. Deficient peritoneal surveillance has been shown to correlate with the existence of peritoneal endometriosis (Ulukus et al. 2008). Indirectly dietary intolerance may therefore encourage the endometriotic peritoneal colonization by retrograde menstrual flow.
This study looked at the 54 women, divided in two groups differentiated by the diagnosis of laparoscopically confirmed endometriosis. A questionnaire was devised to detect the occurrence of gastrointestinal/gynaecological/general symptoms and dietary intolerance in these two groups of women.

Materials and methods

Fifty-four women who complained of gynaecological symptoms and/ or complained of infertility underwent laparoscopy in effort to reach a diagnosis. These women were recruited sequentially into the study so as to avoid bias (age range 20-55 years). The study was conducted at Department of Obstetrics and Gynaecology, St Luke's Hospital and Mater Dei Hospital from 1st January 2005 to 31st December, 2007.
Following the laparoscopy a questionnaire was set up so as to enquire on occurrence of dietary intolerance and gastrointestinal and gynaecological symptoms in these women.

Results (Tables 1-9)

Out of a total of 54 women, twenty-two women were diagnosed to have pelvis endometriosis. The group of women in the endometriosis group were significantly younger than the other group The gynaecological and

past obstetric history did not reveal any significant differences between both groups. Due to the small number of events occurring within each variable, the groups were compared by category of events i.e. gastrointestinal symptoms, dietary intolerance, general and gynaecological symptoms.
When all the gastro-intestinal symptoms were collated together, these symptoms were more commonly found in the endometriosis group (53%) as compared to the other group of women (31%). Similarly more women with endometriosis complained of intolerance to one or more dietary components (26% versus 14%). These differences did not reach statistical significance possibly because the study was not adequately powered to show this. No significant differences were noted for the other parameters such as infertility, gravidity/parity, gynaecological and general symptoms.

TABLE 1 - CHARACTERISTICS OF PATIENTS RECRUITED FOR THIS STUDY.

	Number of Patients	Age	Laparoscopy	Laparotomy
No Endometriosis	34	44.6±9.2	32	2
Endometriosis	23	39±10.2	21	2

TABLE 2 - REASONS FOR LAPAROSCOPY.

	No Endometriosis	Endometriosis Group
Dysmenorrhoea/Dysparuenia	12	10
Ovarian Cyst	4	1
Infertility	10	8
Pain	8	4
Total	34	23

TABLE 3 - GYNAECOLOGICAL HISTORY OF WOMEN RECRUITED IN STUDY.

	Number of Patients	Menarche	Regular cycles	Irregular Cycles	Duration	Menstrual Cycle
No Endometriosis	34	12.2	27	5	5.8	30.4
Endometriosis	23	12.4	20	2	4.7	26.2

TABLE 4 - GASTROINTESTINAL SYMPTOMS IN BOTH GROUPS OF WOMEN.

	Number of Patients	Abdominal Pain	Constipation	Diarrhoea	Bloating	Flatulence
No Endometriosis	34	23	14	5	13	14
Endometriosis	23	22	18	9	9	9
	Number of Patients	**Flatulence**	**Heartburn**	**Indigestion**	**Belching**	**Mucus in stools**
No Endometriosis	34	14	9	5	6	0
Endometriosis	23	9	19	14	4	2

TABLE 5 - DIETARY INTOLERANCE TO VARIOUS TYPES OF FOOD.

	Number of Patients	Bread	Pizza	Pasta	Red Meat	White Meat
No Endometriosis	34	9	9	5	0	2
Endometriosis	23	10	10	5	3	0
	Chicken	**Fish**	**Dairy Products**	**Milk**	**Yoghurt**	**Vegetables**
No Endometriosis N=34	1	1	5	4	1	8
Endometriosis N= 23	0	0	8	7	3	11

TABLE 6 - PAST OBSTETRIC HISTORY.

	Number of Patients	Gravidity/woman	Parity/woman	Miscarriages /woman
No Endometriosis	34	2.2	1.9	18
Endometriosis	23	2.1	1.9	9
	Number of Patients	**Infertility**	**Duration of Infertility (years)**	**Primary Infertility**	**Secondary Infertility**
No Endometriosis	34	13	3.6	3	10
Endometriosis	23	8	4	6	2

TABLE 7 - GYNAECOLOGICAL HISTORY.

	Mild Dysmenorrhoea	Moderate Dysmenorrhoea	Severe Dysmenorrhoea		
No Endometriosis N=34	12	6	5		
Endometriosis N=23	11	6	6		
	Mild PMS	**Moderate PMS**	**Severe PMS**	**Superficial Dysparuenia**	**Deep Dyspareunia**
No Endometriosis N=34	10	7	4	4	4
Endometriosis N=23	14	3	4	7	9

TABLE 8 - GENERAL SYMPTOMS.

Number of Patients		**Wheezing**	**Hypothyroid**	**Depression**	**Numbness**
No Endometriosis N = 34		9	4	7	14
Endometriosis N = 23		9	2	13	8
Number of Patients	**Painful joints**	**PID**	**Drowsiness**	**Irritability**	**Poor Coordination**
No Endometriosis N = 34	14	6	13	17	7
Endometriosis N = 23	12	3	9	14	6
Number of Patients		**Mood Swings**	**Headaches**	**Muscle ache**	**Muscle weakness**
No Endometriosis N = 34		14	25	15	4
Endometriosis N = 23		15	14	14	7
Number of Patients		**Dizziness**	**Swollen fingers**	**Itching**	**Rashes**
No Endometriosis N = 34		14	10	11	0
Endometriosis N = 23		9	7	4	2

TABLE 9 - COMPARISON OF OCCURRENCE OF EVENTS BY CATEGORY IN BOTH GROUPS OF WOMEN.

	N	**Gastrointestinal Symptoms**	**Dietary Intolerance**	**Gynaecological Symptoms**	**General Symptoms**
No Endometriosis	34	89	45	82	190
Endometriosis	23	105**	57*	69	149
		p= NS	P=NS	p=NS	p=NS

Discussion

This study reviewed a large number of variables between two groups of women who required laparoscopy so as to establish a diagnosis for a variety of gynaecological complaints. The two groups were differentiated by the presence of pelvic endometriosis which was found in twenty-three of the fifty-four women recruited. Following the laparoscopy a large number of symptoms were broadly categorized into gastrointestinal symptoms, gynaecological complaints, general symptoms and enquiry on dietary intolerance to a variety of foods was asked in a comprehensive questionnaire.

The results were assessed on individual symptoms and according to symptom category. In view of the large number of individual complaints the parameters were statistically examined according to category. The two categories covering gastrointestinal symptoms and dietary intolerance showed nonstatistically different results when comparing both groups of women. When comparing gynaecological complaints and general symptoms no statistical difference was borne out.

Gastrointestinal symptoms and dietary intolerance (in the main gluten and lactose based) are obviously linked and this finding may explain the above findings. It is interesting to note that both variables showed the same trend in the group of women with endometriosis. This suggests a connection possibly due to an underlying immunological cause that may be relevant to the pathogenesis and may possibly shed light on a more effective management of endometriosis.

Current thinking supports the initiating mechanism to peritoneal endometriotic colonization to be due to retrograde menstrual flow as expounded by Sampson. However, since retrograde menstruation occurs in most women of the reproductive age, it is clear that

there must be other factors which may contribute to the implantation of endometrial cells and their subsequent development into endometriotic disease. Following the retrograde menstrual flow a number of immunological mechanisms have been implicated. There is a substantial body of evidence to support that the alterations in both cell-mediated and humoral immunity contribute to the pathogenesis of endometriosis (Ulukus et al. 2005).

Alterations in cellular immunity may result in inadequate removal of ectopic endometrial cells from the peritoneal cavity. This may be deduced by the increased number and activation of peritoneal macrophages and the paradoxically decreased T cell and natural killer cell cytotoxicities in the presence of endometriosis natural killer activity and the cytotoxicity against autologous endometrial cells have been shown to decrease in women with endometriosis and correlate with the severity of the disease. Decreased cytotoxicity to endometrial cells in women with endometriosis is therefore thought to be due to a defect in natural killer activity but is also partially because of a resistance of the endometrium to natural killer cytotoxicity (Oostelynck et al. 1991). Polymorphisms in killer cell immunoglobulin-like receptors may be responsible, in part, for genetic susceptibility to endometriosis. The frequency of killer cell immunoglobulin-like receptors (KIR3DS1) has been shown to be s significantly decreased in patients with endometriosis compared with controls (32% versus 44%, P=0.028). These results suggest that polymorphism in killer cell immunoglobulin-like receptors may be associated with susceptibility for endometriosis (Kiwatawa et al. 2007).

Increased levels of several cytokines and growth factors which are secreted by either immune and endometrial cells seem to promote implantation and growth of ectopic endometrium by inducing proliferation and angiogenesis. Immunoreactivities of both chemokines interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) have been shown to be significantly increased in the epithelial cells of ectopic endometrial tissues compared with those of normal endometrium. These findings suggest that IL-8 and MCP-1 may be involved in the pathogenesis of endometriosis (Ulukus et al. 2008). Serum levels of MCP-1 were found to be significantly higher in patients with endometriosis. These results imply the potential of MCP-1 measurements for the diagnosis of endometriosis (Agic et al. 2008).

Tumour necrosis factor alpha of peritoneal fluid is believed to have important pro-inflammatory and angiogenic activities in the complex mechanisms of development of peritoneal endometriotic lesions. Impairment of macrophage function supports the theory that an inappropriate immunological reaction of the peritoneal milieu to retrograde ejected endometrium may play a part in the initial phases of endometriotic implants (Calhaz-Jorge et al. 2000).

In addition to the impaired capacity of the immune cells to mediate endometrial cell removal, inherent resistance of the ectopic endometrial cells against immune cells is another interesting concept in the pathogenesis of endometriosis. In the initial stages of endometriosis the activated peritoneal fluid natural killer cells can be intensively eliminated, thus providing conditions for the survival of ectopic endometrial cells and the development of the disease (Eidukaite et al. 2006). Sera from patients with endometriosis suppressed natural killer cell zeta expression, which resulted in suppression of these cell interferon gamma induction (Bohler et al. 2007). Increased expression of CD94/NKG2A in peritoneal natural killer cells may mediate the resistance of endometriotic tissue to natural killer cell-mediated lysis, thus contributing to the progression of endometriosis (Galandrini et al. 2008).

Endometriosis has also been considered to be an autoimmune disease, since it is often associated with the presence of autoantibodies, other autoimmune diseases, and possibly with recurrent immune-mediated miscarriage. Patients with endometriosis also exhibit autoantibodies reactive with cellular proteins; endometrial membrane proteins exhibited the greatest reactivity, followed by nuclear antigens. A spectrum of auto-immunity has been noted when in subcellular fractions, patients with stage III endometriosis exhibited significantly more immunoreactivity than did stage II patients, which was greater than that observed in stage I patients (Bohler et al. 2007).

Immune reactions similar to those found in endometriosis have been shown with dietary proteins such as gluten and casein may result in alteration in both humoral and cell mediated immunity. In particular, similar cytokines and T cell reactivity have been noted in endometriosis and oral dietary gluten and lactose intolerance. Abnormal immunity to foods, due to inappropriately high titers of antibodies or qualitatively altered cell mediated responses such as natural killer cells, produce disease. This may be epidemiologically significant its prevalence in some populations, for example of lactase deficiency, with dose-related lactose and milk-intolerance, occurring in as much as 50-90% of most populations. (Ferguson 1995).

Immunoregulatory invariant natural killer cells rapidly produce interleukin (IL)-4 and other cytokines that suppress a Th1 response and are deficient in some autoimmune diseases and this deficiency is thought to also influence the immunoregulatory natural killer T-cells in gluten enteropathy. Natural killer T-cells in coeliac subjects were shown to be reduced to 30% of those in normal subjects. These findings sug-

gest that immunoregulatory natural killer T-cells are deficient in celiac disease (Grose et al. 2007, Grose et al. 2008).

Ingested gliadin, the triggering agent of the disease on crossing the epithelial barrier may elicit a harmful T cell-mediated immune response. This seems to be mediated through an increased secretion of chemokines and cytokines mainly of IL-6, IL-8, IL-10, TNF-alpha, growth-related oncogene, MCP-1 and macrophage-derived chemokine. Maturation was accompanied by a greater capacity to stimulate proliferation of allogeneic T cells and significantly reduced endocytic activity (Palova-Jeninkova et al. 2005).

As regards milk intolerance, lactose has been shown to block galectin-3 is a beta-galactoside-binding protein implicated in diverse biological processes. Galectin-3 induces a human monocyte migration in vitro in a dose-dependent manner, and it was chemotactic at high concentrations but chemokinetic at low concentrations (Sano et al. 2000).

A lactose-binding protein, MCF-pl5-L exhibited an evident dose-dependent monocyte chemotactic activity for monocytes and macrophages. The biological functions of MCF-pl5-L include prolonging the life span of macrophages, probably by inhibiting apoptosis of macrophages, and stimulate the production of TNF-alpha from macrophages (Yamanaka et al. 2000).

The immunological pathways of both endometriosis and food intolerance are undoubtedly protean as evidenced by the abundant and complex literature currently available. It is not unreasonable that however complex these immunological pathways are they may each other's path including in the initiation of disease. The findings in this study may lend support to this hypothesis with the significant preponderance of gastrointestinal symptoms and frequency of dietary intolerance noted in the women diagnosed with endometriosis.

Undoubtedly further research in this direction is required. However if a connection between endometriosis and dietary intolerance is indeed proven this may have a favourable impact on the deduction of the pathogenesis of endometriosis and possibly a useful adjunct in the treatment of this enigmatic disease.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Hormonal replacement therapy in women with menopausal symptoms and evaluation of the effects on cardiovascular system

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Introduction

At present most investigators consider hormonal replacement therapy (HRT) in peri- and postmenopause pathogenetically proved as it provides adaptation of women's organism to a new metabolic balance as a result of ovarian failure (1). But various types of HRT, different in the way of drug effect, especially progestin effect, HRT regimens, dose schedule may have different effects on cardiovascular system. Most progestins found in HRT combinations are third generation agents. In the present study was used HRT, containing drospirenone, a spironolactone analogue, which has a unique pharmacologic profile that is similar to that of natural progesterone (2). It combines progestogenic, antimineralocorticoid properties and has virtually no androgenic activity. As a component of HRT regimen its antimineralocorticoid effects may prevent weight gain secondary to water retention and the decrease of blood pressure (3). The prospective, controlled cohort study EURAS-HRT (European Active Surveillance Study of Women taking HRT) showed the decrease in systolic blood pressure and stable body weight in the E2/DRSP group and these effects were consistent with drospirenone anti-aldosterone properties (4). Thus, the objectives of our study were to estimate-menopause-related symptoms and consider the influence of HRT on blood pressure (BP), waist circumference, body mass in postmenopausal women.

Materials and methods

This prospective study included examination of 170 postmenopausal women, average age was 51.0±5.0 years. All women underwent clinical, laboratory, ultrasound, X-ray and cytological examination. It is known that after menopause women have a tendency to develop different components of metabolic syndrome which to a certain extent explains the cardiovascular risk increase. That is why we estimated anthropometrical parameters, including weight, body mass index (BMI) and waist circumference (WC). While questioning patients we used Kupperman menopausal index, Spielberger test assessing personality and reactive anxiety, Beck scale assessing the presence and expressiveness of depression and eating behavior tests (emotional, external, restrictive). The 24-hour ambulatory-blood pressure monitoring data and officeblood pressure measurement were also determined. The study lasted 12 months. According to the results of anthropometrical measurement the average waist circumference was 84.5±13. 4sm, average BMI was 27.3±1.4 kg/m^2 and average weight was 73.5±14.1 kg. Average systolic blood pressure (BP) before the therapy was 140.8±5.0 mmHg and diastolic BP was 77.9±5.0 mmHg. Different menopausal symptoms included: hot flushes-72.3%, joint and muscular discomfort - 69.5%, sexual problems-70.1%, dryness of vagina-68.9%, physical and mental exhaustion-50.7%, irritability-70.1%, insomnia-43.6%, depression- 30.4%, heart discomfort-35.1%. According to the results of the examination women who had indications and without contraindications, willing to get HRT, were prescribed 1mg 17-beta-estradiol and 2mg drospirenone. Exclusion criteria were: arterial hypertension stages 2 and 3, secondary (symptomatic) hypertension, coronary heart disease, territorial stroke or transient ischemic attack in anamnesis; diabetes mellitus 1 and 2 types; comorbidities demanding additive therapy and

making difficult the evaluation of drug effectiveness and tolerance, severe hepatic disorders, verified or supposed diagnosis of breast cancer, verified or supposed diagnosis of premalignant estrogen-depended disease or estrogen-depended cancer; history of venous and arterial thrombosis and thrombembolia; unknown bleeding from genital tracts; hypertriglyceridemia (>6 mmol/L).

Results

We noted a general decrease of the manifestation degree of complaints in women, who were prescribed HRT ($p<0.05$), after 12 months. HRT resulted in 40% decrease of the sympathetic nervous system tonus, associated with the decrease of systolic blood pressure (SBP) and diastolic blood pressure (DBP) by 8.7 mm.Hg and 5.3 mm.Hg respectively ($p<0.001$). The HRT, containing 1mg 17-beta-estradiol and 2 mg drospirenone was efficacious in the treatment of menopausal disorders ($p<0.005$). After 12 months-there was observed the decrease of psycho-emotional, vegetative-vascular, metabolic-endocrine disorders. The average grade of vegetative-vasculardisorders was 3.3 ± 0.5, psycho-emotional disorders was 1.6 ± 0.3, metabolic-endocrine disorders was 1.1 ± 0.2($p<0.05$). Mild degree of vegetative-vascular disorders was present in 3% women, and 97% had less than 10 grades, i.e. vegetative-vascular disorders were practically absent. 50% women had milddegree of psycho-emotional disorders, 3.1% had meandegree, a severe degree of symptoms was absent. Metabolic-endocrine disorders were of mild degree and were present in 90% of women. The average gradewas 1.1 ± 0.2. Our study demonstrated positive interactions between HRT prescription and significant decrease of SBP and DBP. The HRT has a favorable influence on the body mass and waist circumference. Waist circumference decreased by 1.7 sm (12% of initial value), weight decreased by 3.3 kg. BMI decreased from 27.3 ± 1.4 kg/m^2 to 25.6 ± 1.2 kg/m^2. The side effects were minimal, of temporary character and did not influence therapy compliance.

Discussion

Climacteric is one of the major risk factors developing CVDs in middle-aged women (5,6,7). And though menopause is not a disease, it leads to the impaired endocrine balance in the organism contributing to CVD developing. The main way to correct the climacteric disorders is HRT prescription. The effectiveness of HRT according to data of different studies ranges from 70 to 90%. Many authors recognize that the effect from HRT varies concerning different symptoms of climacteric syndrome, and is dependent on the characteristic of present complaints and components of the drug. After the menopause begins, women have a tendency to develop different components of the metabolic syndrome which to a certain extent explains the cardiovascular risk increase. Weight gain is frequent in postmenopausal women. Particularly, the menopause is associated not with the increase of subcutaneous fat, but with the developing visceral obesity (intra-abdominal visceral fat, epicardial adipose mass, liver fat). It is now well- established that abdominalcircumference, which is used as a surrogate marker of visceral fat, is an easily available clinical measurement of both cardiovascular risk and type 2 diabetes risk in obesity. An expanded visceral fat mass, when measured as waist circumference, is a better predictor of insulin resistance, dyslipidemia, low-grade inflammation, and other risk factors for both type 2 diabetes and cardiovascular disease than obesity measured with body mass index. Besides, obesity is associated with the BP increase, especially SBP that is associated with a higher cardiovascular risk than DBP in women with arterial hypertension (8). Studying the impact of HRT on cardiovascular system and metabolic parameters will enable to use it wider and more rationally in complex therapy of climacteric syndrome in postmenopausal women. The differentiated approach of hormonal therapy with the integrated evaluation of the quality of life, emotional background, expressiveness of climacteric syndrome, status of cardiovascular system is very important and timely.

Conclusion

In conclusion, the HRT prescription, containing 1mg 17-beta-estradiol and 2mg drospirenone, showed a significant decline of menopause disorders and a significant decrease of SBP and DBP. Positive changes with the HRT prescription are more expressed in women with initial metabolic disturbances.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Influence of combined oral contraceptive pill containing Drospirenone 3mg and Ethinylestradiol 20mcg on circadian arterial stiffness indicators in perimenopausal women

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Introduction

Among traditional risk factors, associated with increased arterial stiffness, the age-related estrogendeficiency is particularly important. Cardioprotective effect of female hormones is determined by their beneficial impact on lipid profile changes (1), improvement of the endothelium functional condition, suppression of vascular smooth muscle cell's proliferation, the decrease of collagen production in vascular wall in a response to injury, blocking slow calcium canals and antimineralcorticoid activity, the decrease of inflammatory processes associated with atherogenesis, the suppression of procoagulation activity (2,3). With hormonal changes, occurring in menopause, special clinical features, characteristic of early climacteric period, begin to appear: psycho-emotional, vegetative-vascular, metabolic-endocrine disorders (4,7). Because of estrogen deficiency the balance of vasoactive mediators is impaired with the predominance of vasoconstriction production and vasodilatation secretion decline, reported increased collagen production in vascular wall that initiates processes of vascular remodeling and results in the arterial stiffness increase. Presumably, early prescription of combination hormonal therapy, containing estradiol and drospirenone in premenopausal women could have a beneficial impact on arterial stiffness. The aim of this work was to study the dynamics of indicators, characterizing circadian arterial stiffness, in perimenopausal women and its variation during combined oral contraception containing drospirenone 3 mg and etinilestradiol 20 mcg in take.

Methods

The study included 65 women: group 1 consisted of 37 women in premenopause with climacteric symptoms who were prescribed combined oral contraceptive containing drospirenone 30mg and ethinylestradiol 20 mcg, average age was 49.2±2.6years, and control group 2-28 women in perimenopause, average age was 53.3±5.1 years ($p<0.001$), who didn't take oral contraceptives. In group 1-6 women (16.2%) had obesity stage 1 and 3 women (8.1%) with stage 2. In control group 2: 6 women (21.4%), 4 women (14.3%) and 2 women (7.1%) had obesity stage 1, 2 and 3 respectively. Duration of hypertension stage 1 was on average 1.2±1.1 years in group 1 and 1.3±1.1 in group 2. Body mass index (BMI) was 27.3±5.2 kg/m^2, waist circumference (WC) was 85.5±12.9sm in comparison with group 2: BMI was 30.5±5.7kg/m^2, WC was 95.4±14.8sm ($p<0.01$) respectively. The study lasted 12 months. All patients underwent clinical examination, including the anthropometrical measurement, 24-hour ambulatory blood pressure monitoring (ABPM) with determining pulse blood pressure (PBP), arterial oscillometry for vascular stiffness evaluation. During arterial stiffness examination there were estimated pulse transit time (PTT2), augmentation index (AIx,%), arterial stiffness index (ASI) and ambulatory arterial stiffness index (AASI), modified indices PTT100-60, ASI100-60 AIx-75. All women underwent ECHO-KG with determining aortic pulse wave velocity (PWV). Unlike aortic pulse wave velocity, as a more direct indicator, characterizing the arterial stiffness, such values as augmentation index, arterial stiffness index of the brachial artery and ambulatory arterial stiffness index are indirect indicators of arterial stiffness (6).

Results

In the study we found that BP dynamics with the beginning of the menopause has its particular characteristics

–predominant systolic blood pressure (SBP) increase, higher PBP level, insufficient SBP and diastolic blood pressure (DBP) nocturnal decrease, higher BP variability. According to the results it was established that arterial stiffness is a dynamic characteristics and the dynamics of indicators shows the decrease of arterial elastic properties in women during menopause. Vascular elastic properties differ along the arterial tree – proximal arteries are more elastic than the distal ones and, consequently, PWV differs a lot in various regions of the arterial bed. It was shown that normal PWV rate varies from 4 to 6 m/s and in arteries of muscular type it increases to 8-12 m/s (6).

After 12 months PWV mediana was significantly higher in group 2: 7.2 (5.9;8.3) m/s in group 1 and 6.4 (5.7;7.3)m/s in group 2 respectively ($p<0.05$). Significant increase of PBP was marked in group 2: 49.5(42.0;56.0) mm.Hg versus group 1: 43.5(39.0;45.0) mm.Hg ($p<0.05$). PBP is determined as an interaction between left ventricular contractile function and expansibility of magistral arteries (direct component) and the value of the reflected wave (indirect component). The PBP and SBP increase is associated with the elevation of arterial stiffness and accompanied by the increase of wave reflection amplitude. PBP is an independent factor of cardiovascular events and demonstrates the real arterial age (5,6). The increase of AIx-75 was observed in 39.2% women in group 2 and in 10% in group 1 ($p<0.05$) respectively. AIx-75 is a modified index, characterizing the expressiveness of the reflected wave and its contribution to the PBP increase. Main factors, determining this parameter, areaortic rigidity and peripheral resistance.

The value of AIx can potentially be considered as a criterion of cardiovascular risk. In perimenopausal women higher ASI100-60 – 125.0(110.0;160.0) c.u. was observed compared with group 1 - 114.0 (104.0;124.0) c.u. ($p<0.05$). ASI is a dynamic parameter, reflecting arterial stiffness and depends on structural changes in arteries, BP level and vascular tone. At present the significance of this parameter continues to be specified, but recent data points to its possible prognostic role in cardiovascular risk evaluation. At the moment of the research termination there was marked the decrease of pulse blood pressure (PBP) to 1.2 mm.Hg, modified index AIx-75 to 1.2%, ASI on 10.2 c.u. ($p=0.06$), AASI on 0.04 and the increase of modified index PTT2100-60 on 3.8 ms. Women in control group 2 had the opposite changes: PBP increase by 2 mm.Hg, AIx-75 by 7.4% ($p=0.06$), ASI by 9.7 c.u., AASI by 0.02 c.u. and significant decrease of daily average modified PTT2100-60 by 10.5 ms ($p<0.01$). PWV significantly decreased by 0.2m/s in group 1 and increased by 0.2m/s in group 2 ($p>0.05$). In comparison with daytime it was mentioned that in nocturnal hours there were determined significantly higher values of Aix, a distinct tendency to the ASI, modified AIx-75 and ASI100-60 increase, significantly less values of PTT2100-60. According to the results of the study there were detected interactions between PWV and main studying parameters. There were determined moderate correlations between PWV and PBP ($R=0.43, p<0.001$), PWV and AIx-75($R=0.35, p<0.01$), PWV and PTT2100-60 ($R=-0.5, p<0.0001$), average daily SBP variability ($R=0.57$, $p<0.00001$) and DBP ($R=0.60$, $p<0.0001$). However such parameters as ASI and AASI had weak correlation with PWV and, increasingly, had interactions with average daily BP parameters. There were detected moderate correlations between SBP and ASI100-60 ($R=0.53$, $p<0.0001$), PBP and AASI ($R=0.64$, $p<0.0001$), DBP and AASI ($R=0.32$, $p<0.05$). During the analysis of relative correlations between AIx, ASI and AASI, they were revealed only between AIx-75 and AASI ($R=0.45$, $p<0.0001$).

Conclusion

Perimenopausal women in comparison with premenopausal women have significantly increased indicators, characterizing circadian arterial stiffness, and early prescription of combined oral contraceptive, containing drospirenone 30 mg and ethinylestradiol 20 mcg in premenopausal women can supposedly prevent its increase. Most informative indicators in evaluation according to the results of our study were PWV, PBP, and modified indices PTT100-60, ASI100-60 AIx-75. It is reasonable to analyze all these indicators in combination as they reflect various aspects of arterial stiffness.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Regulation of thyroid receptors mRNA and protein expression by hyperthyroidism in corpus luteum of rats in late pregnancy

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Introduction

Thyroid hormones (THs) play critical roles in differentiation, growth and metabolism. THs are needed in an adequate concentration to support CL formation and pregnancy.
We have found that experimental hypothyroidism in pregnant rats produces a delay in luteolysis and parturition.
Conversely, hyperthyroidism (hyperT) advances the decrease in circulating progesterone and parturition by approximately 12 hours.
The classic genomic action of THs are mediated by nuclear receptors (TR) acting as hormone inducible transcription factors.
The TRα1, TRα2 and TRβ1 isoforms are widely expressed whereas TRβ2 is mostly restricted to the hypothalamus-pituitary axis. TRα1, α2 and β1 mRNA and protein are present in ovarian surface epithelial cells in human, while human oocyte, granulose and cumulus cells also express TRβ2 in addition to the other isoforms.
Although at present the presence of TRs in corpus luteum has not been confirmed, we have found that experimental hypothyroidism/hyperT delay or advance luteolysis and parturition in rats. Thus, thyroid hormones affect luteal function.
In order to establish the existence of luteal TRs, we determined which isoforms of thyroid receptor are expressed in rat corpus luteum by real time PCR and Western-Blot and if these proteins are regulated by experimental hyperthyroidism.

Materials and methods

For this purpose, groups of Co (control) and hyperT (induced by daily injection of 0.25 mg/kg of T_4) Wistar rats (n= 6-8) were sacrificed at day 19 (G19), 20 (G20) and 21 (G21) of gestation. Total CL RNA and proteins were prepared using TRIzol. Luteal TR mRNA was measured by real time RT-PCR and expressed relative to the expression of the housekeeping gene rat ribosomal protein S16 cDNA. Proteins, isolated from the phenol-ethanol supernatant obtained from RNA isolation, were analyzed for TRα1 and TRβ1 abundance by Western blot and expressed as the ratio of signal intensity for the protein relative to that of b-tubulin.

Results and conclusions

Corpus luteum of pregnancy expresses TRα1, TRα2 and TRβ1 mRNA. There were no changes in TRα1 and TRα2 expression between days and groups studied. TRβ1 expression was similar in the three days in controls and was increased in hyperT rats on G19 and G21. TRα1 and TRβ1 protein decreased significantly in hyperT rats on G20. This decline in TRα1 and TRβ1 protein can be due to degradation at the time of functional luteolysis that occurs in this day in hyperT rats. These results show that both isoforms of TRs are present at luteal level, that they are sensitive to regulation by thyroid hormones and that there may be a dissociation between TH regulation in the expression at mRNA and protein levels.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The climacterics and menopauses: paraphysiological bio-neuro-endocrine evolution

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Introduction

There is a physiological interaction, functional and protective action between neurotransmitters and hormones that have not yet been fully codified. The dynamics of these metabolic substances is dependent on changing endogenous and exogenous factors. The same potential and variability of expression (demand-response) produce different systemic inductions we call para-pathological situation determining a reduced quality of life of every woman in menopause. These events are coded and predictable only if you have a comprehensive understanding of the female body. It would thus produce a coherent response to these functional and therapeutic events of brainstorming that have resulted not only as a cognitive function and systemic body decrease but also in terms of drugs and specialistics consulting, giving an inevitable loss of quality of life.

Aim of the study

From the time that the ovarian reserve ceases upon cessation of the menstrual cycle in an irreversible situation, you have two bio-neuro-hormonal definitions: *peripheral climateric* and *central climateric*. They are followed by three different conditions of the same situation as defined: *limbic menopause, hypothalamus menoapuse* and *central menopause syndrome.*

Observations and deduction

Those frameworks, different from both clinical and symptomatic point of wiev are independents units from hormonal impregnation and need custom therapy to stabilize symptoms properly.
Female neuro-endocrine axis need a stimulating and protecting action from the estrogen on the direct pituitary and hypothalamic gland.
Each step and especially the *bio-processing of signals* from *chemical* (neuro-hormone cytokines) to *electrical (neuro plasticity* and *dielectric phasic potential*) and the resulting effector response depends on hormone conditions that predisposes to the possible neuro-biological response due to the estrogen presence.
In turn, the sex hormone acts at the level of neuronal cells in different ways by interacting with modulatory responses in microglia and neuronal astroglia in different ways depending on the pro-inflammatory state.
The concept of general knowledge is essential as the metabolic state of the body exerts a central question in this report answer: from inflammation of excretory organs, connective, enteric (dysbiosis or candidiasis) recruited through direct paths of the lymphatic spread of blood cytokines and chemokines dissemination, mycotoxins or fractions that are viral tropism are highest in the cerebral cortex (the brain is the only organ that has no potential to accumulate glycogen but extremely limited in time and needs a steady flow through the metabolism of cholesterol, triglycerides above).
So this inflammatory event has its maximum expression at both central and peripheral neurons. Predisposed to neuro-biological response due to the presence of estrogen (Table 1).

TABLE 1.

Defect production	Metabolic defect	Defect-induced	Receptor defect
Ineffective response	Variable response	Answer blocked	Desensitization

The endocrine system is not able to be modulated independently, only through a pure chemical interference factor (hormone-receptor-effector-hormone) but through a great transformation of electrical messages that are translated from the hypothalamus (electroneurochimica) in chemical substances (neurotropic) which in turn are transformed through a process of translation of-chemical hormones by the anterior pituitary lobe and from there superspecifica induce production of factors Realising hormons (ACTH, TRH, etc.). This will produce target organs for a chain reaction back to the periphery to the CNS which will regulate productivity itself.

This process explains the process of circuit corticalized information devices and their endocrine control. To all this we add that any alteration in this process can cause physiological stress response. Endocrinological studies show that a stress condition can interfere directly on neuro-endocrine female body as the main name plate but not the reproductive organs through the adrenal glands that produce cortisol ACTH reactive interference. Cortisol, in turn, causes a direct negative feedback towards the hypothalamus and anterior pituitary lobe that consequently the produce the same modulation of estrogen.

The same stimulus depending on the stress-neuronal cells (microglia, neurons, astrocytes) with which it interacts has a release of substances in response to chemokines and cytokines stimulating a systemic inflammatory process. Consequently, depending on the cell phone off again possible, neuro-plasticity, physiological and metabolic capacity of connective tissue apoptosis is a "restitutio ad integrum" of the system but at the time of menopause in the hormonal storm in the first period of post-menopausal such an event may be exacerbated, unstable and reversible if recognized!

This is why women already in menopause and post-menopausal women may develop other symptoms attributable to peripheral or central involution of the central nervous system and metabolism. In Tables 2 and 3 characteristic symptoms of the various types of events have been coded.

TABLE 2.

Climateric Periferic Synd. =>	CPS Climateric Central Synd. => CCS
Hormone deficiency / reproductive	Increased aggression/Hyperexcitability
Anxiety / Insomnia	Small panic attacks
Headache / Migraine	Lite Hot flashes / sweats
Reduced Libido	Hinstability Hypertension
Mood Swings	Increased vascular tone
Depressive State	Weight gain weight
Loss of Concentration / Memory	Digestive disorder

TABLE 3.

Menopause Limbic Synd.=>MLS	Menopause Hypothalamus Synd. => MHS	Menopause Central Syndrome => MCS
Anxiety / Insomnia	Increased aggression	Ipoergia
Mood Swings	Hyperexcitability	Altered sleep-wake rhythm
Headache / Migraine	Hot flashes / sweats	Progressive cachexia
Depressive State	Hypertension	Progressive loss of mental agility
Reduced Libido	Increased vascular tone	Cardio-pulmonary disease
Loss of Concentration / Memory	Weight gain weight	Multiple dysmetabolism
Alzheimer Type Dementia	Digestive disorders	Intestinal malabsorption / intolerance multiple / allergies

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

SAT-Therapy® in menopausal syndrome

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Introduction

From the last research on the alfa and beta estrogen breast receptors specificity, have been see a possible interaction using soy isoflavons derivates. Conversely the isoflavons derivates of trifolio pratensis have a small reaction only to the beta helpless receptors for the breast. All this new information limits the possibility of phytoderivates prescription and create the need to use other pharmaceutical products, specially made for the menopausal symptoms, respecting the woman body physiology.
So, for the control of the menopausal syndrome, to propose and to use other standard therapeutical choice, controllable, reproducible and therapeutical functionally, as well stable in the future, result "modern".

Background

Considering that is not longer allowed to talk "generally" about menopause, the symptoms, sometime highly disabling for women, can be grouped in two separate types: Menopause Limbic Syndrome and Menopause Hypothalamus Syndrome (will see the differences); those assume specific connotations and need therapeutical customizations to stabilize the cohort of symptoms.
Considering this, we can understand the failure of some unique replacement therapeutical proposals for both Syndromes. This is the starting point of our innovative research and therapeutical application.

Materials and methods

SAT-Therapy® is know for a long time ago, even in science, as support as neo-adjuvant, adjuvant and coadjuvant in various fields such as immunology, endocrinology and oncology, in situation as side effects from radio and/or chemotherapy it has any replacement function, neither active ingredients that can destabilize tissue and organ function physiological balance!
Certified free of side effects. Hormone-free. Derivative-free tissue.
It is based on "fine" selection of antibody fractions (FAB) affinity for specific tissue (confirmed by immunofluorescence studies by the end of last century) who do not have any direct interference in the internal system function, but rather aimed at mobile business rules induced by "activity" of selective protein specific cell membrane (g protein). This level of molecular engineering was produced based on scientific studies repeatable and stable over time. The certification of the quality of the active ingredients produced is is granted by the Pasteur Institute of Paris with anti-viral and anti-prion controls.
150 women aged from 48 to 60 years have been recruited.
Recruitment examinations were evaluated the general clinical parameters with routine sero-sanguineous check.
The subjective symptoms reported at recruitment were selected with yes or no questionnaires:

– 43 pt (28.67%) had already made TOS for 5 years, then stopped it. Without any other opportunity they come back to the starting symptoms;
– 70 pt (46.67%) had already tried a number of

herbal or homeopathic variety but with small or no stable results;
– 37 pt (24.67%) were approaching menopausal syndrome for the first time and did not want to take HRT.

Were assessed for symptoms and menopausal clinic and recruited in two separate protocols.
Have been settled the following protocols:
MLS=> Monday EMONC-TR® / Tuesday DIEN-PH® alternating with SYM-TO® / Wednesday COR-TX® / Thursday NEU-VAS®
mode: 3 following weeks, followed by 1 week off, for 6 cycles corresponding at 6 months
MHS => Monday EMONC-TR® / Tuesday NEUGLAN-F® / Wednesday FO-E® alternating with DV-PF® / Thursday NEU-VAS®
mode: 3 following weeks, followed by 1 week off, for 6 cycles corresponding at 6 months.
The active ingredients used correspond to the following functional characteristics of relevance:
EMONC-TR® => functional stimulation of multi excretory organs for a body global drain
works on: intestine, liver, kidney, pancreas, reticulo-endothelial system, gall bladder, lungs, skin, lymph nodes.
Directions: during metabolic diseases / with complex therapies/ that weighed down with side effects / restoration of organic response / to module or restore the functionality and metabolism of the above mentioned parts
DIEN-PH® => functional regulation of psycho-neuro-endocrine TOP neurovegetative system, "key point" of the cascade, contains diencephalon
SYM-TO® => addressed with remodeling TOP psycho-neuro ... . from the "key point" of the cascade ...
NEUGLAN-F® => functional adjusting of the women and men psycho neuro-endocrine axis, includes: ovarian-pituitary-diencephalon-front; thyroid and surrenal ghlands
COR-TX® => regulation of cortical brain trophic, metabolism and nerve conductance in support of glia neurogenic
NEU-VAS® => regulation of neuro-vascular trophic. Protein fractions derived from nerve-vessel-skin-tess.connectives
FO-E® => to support the liver work, in detoxification and overall support the organ provides
DV-PF® => regulation of the hepato-biliary and digestive functional integrate axis, supporting duodenum - gall bladder - pancreas – liver.

Results (Tabs 1 and 2)

After 6 months of treatment, patients were asked to review clinical routine examinations and to report about symptoms at recruitment with yes or no answers.
The clinical data reported do not indicate any change on health status or hormonal after treatment.
The gynecological clinical status was stable with any effects (inflammation of haemorrhoids, fissures, or urogenital dystrophy).
both groups, after a 12 months check, confirm the therapeutic benefits of treatment, but all patients asks medical treatment under medical control to not lose to the overall benefit obtained, even if stable in time!
This is a subjective parameter that was not initially suspected, but is reported by all patients at each control as "fundamental change during treatment".
This is a "general resentment energy", a background

TABLE 1.

Menopause Limbic Syndrome = > MLS 56 pt	before - SAT 37,33% of symptoms related to limb function	After 12 month SAT 23.21% treatment resistant symptoms
Anxiety / Insomnia	56 pt - 100% D / therapies recent = past -> weaning	1 pt - 1,79%
Mood Swings	50 pt - 89,29%	0 pt - 0%
Headache / Migraine	6 pt - 10,70% D / being treated for headaches => weaning	0 pt - 0%
Depressive State	39 pt - 69,64% D / psychological therapy also => weaning	1 pt - 1,79%
Reduced Libido	56 pt - 100%	11 pt - 19,64%
Loss of Concentration / Memory	39 pt - 69,64%	0 pt - 0%
Alzheimer Type Dementia	6 pt - 10,71% D / psychological	0 pt - 0%

TABLE 2.

Menopause Hypothalamus Syndrome = > MHS 99 pt	before - SAT 66% symptoms related to hypothalamic function	after 12 month SAT 16,16% of treatment resistant symptoms
Increades aggression	30 pt - 30,3%	0 pt - 0%
Hyperexcitability	80 pt - 80,81%	0 pt - 0%
Hot flashes /sweats	99 pt - 100%	7 pt - 7,07%
Hypertension	70 pt - 70,71% D / systemic hypertension evaluated by Doppler of 24 hours	9 pt - 9,09% a great reward in classical pharmacological stability
Increased vascular tone	40 pt - 40,4% D / doppler carotid-AAll	0 pt - 0%
Weight gain weight	99 pt - 100%	0 pt - 0% stability or decrease in body weight
Digestive disordes	45 pt - 45,45% D / EGD = reflux esophagitis / gastritis Ab anti HP +	0 pt - 0% 12 months after endoscopy negative

tone of psycho-physical well-being which has nothing to do with the psychological side, but rather with a kind of improvement of psycho-physical and metabolic endurance that causes the therapeutic proposal continue with only 2 cycles of 3 months between October and December and between April and June. data are being developed for long-term period.

Conclusions

The SAT-Therapy®, considering our clinical and psychophysical data, is therefore placed as a valid proposal and choice in the general treatment of menopausal syndrome under the compliance of patients.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Comparison of reproductive outcomes using IVF-ET and ICSI in ART cycles in which only one or two oocytes are retrieved

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Introduction

One of the goals of Assisted Reproduction Techniques (ART) is the recruitment of multiple follicles and the recovery of good quality oocytes.
Ovarian response (OR) can be defined as the reaction of the ovaries to an exogenous stimulation: it varies substantially among women and in the same woman between various cycles (1). Hence, poor ovarian response indicates a reduced follicular response resulting in a low number of retrieved oocytes, despite the high dose of gonadotropins administered.
In recent years there is an increasing number of in vitro-fertilization (IVF) patients in whom few oocytes are obtained in response to controlled ovarian hyperstimulation (COH), mainly due to the postponement of childbearing and the decrease of ovarian reserve. As a consequence, it is important to choose the most appropriate technique of fertilization to maximize the chances of pregnancy in this group of patients, that is really challenging for IVF specialists.
Conventional IVF and intracytoplasmic sperm injection (ICSI) are the two principal techniques used to obtain fertilization.
While conventional IVF implies insemination of oocytes with a determined quantity of spermatozoa, on the other hand ICSI involves injection of a single spermatozoon into a mature oocyte (Table 1).
ICSI is usually indicated when a male factor for infertility exists, but sometimes this technique is preferred also if an age-related infertility is present or if few oocytes are retrieved at pick up.
In Italy, assisted reproduction technology treatments have been regulated since 2004 by Law n. 40/2004, until the decision n. 151/2009 of the Italian Constitutional Court, that addressed the constitutional legitimacy of several provisions of Law n. 40. One of the crucial points of the law 40 was that no more than three oocytes could be inseminated, in order to prevent the formation of unused embryos. All the developed embryos must be transferred into the uterus, and embryo cryopreservation was not allowed. As a consequence, many Italian clinics began to perform ICSI even when sperm quality was suitable for conventional IVF (2).
In comparison with conventional IVF, certain disadvantages exist when oocytes are subjected to ICSI. Beyond the mechanical damage that may be caused to the oocyte during injection, gametes carrying chromosomic alterations -that otherwise would be eliminated by natural selection- might be fertilized (3).
The aim of our study was to compare reproductive outcomes in patients undergoing conventional IVF-ET (Group A) or ICSI (Group B), in whom only 1 or 2 oocytes were retrieved at ovarian pick up.

Material and methods

Study design (Fig. 1)
We retrospectively analysed a total of 75 patients (83 cycles) attending assisted reproductive technologies at the Centre of Reproductive Pathophysiology of the Department of Reproductive Medicine and Child Development of University of Pisa in the period between January 2010 and November 2011. Patients were all poor responders and were included in this study when only one or two oocytes were retrieved during oocytes pickup.

TABLE 1 - PRESENT LITERATURE.

Publication	Type of study	Characteristics of patients	No. of cases and controls	Outcomes reported
Ludwig et al. [4](1997)	Retrospective study	12, 18, and 20 cases treated with 1, 2, and 3 oocytes	50 cycles with <4 oocytes retrieved	Rate of metaphase II oocytes injected and fertilized, and intact oocytes similar in the three groups. Transfer rate not significantly different from those cases with >3 oocytes
Moreno et al.[5](1998)	Prospective study	6 or fewer retrieved oocytes	96 non-male infertile coup les (104 cycles), randomly divided into 2 groups, one by IVF and the other by ICSI.	Similar fertilization rates (FR) per inseminated oocyte and per obtained oocyte. Equal number and quality of embryos obtained and comparable pregnancy (PR) and implantation rates (IR). Neither the number of retrieved oocytes, nor patient age was relevant for the fertilization rates obtained with both techniques. Number of complete fertilization failure similar in both procedures.
Staessen et al.[6] (1999)	Auto-controlled study	Tubal infertility and normozoospermic semen	56 couples randomly divided into 2 groups inseminated either by IVF or by ICSI.	Total cleavage rates were quite similar, no difference in embryo quality. At 42 h post-insemination more embryos were at the four-cell stage after ICSI than after IVF. There appeared to be no difference in implantation potency of the embryos obtained with either technique after the non-randomized transfers.
Fishel et al.[7] (2000)	Prospective randomized study	5 groups of patients,inseminated with husband's sperm atozoa or with donor's	221 patients	ICSI with husband's spermatozoa had a higher FR as compared with IVF or IVF with HIC with donor spermatozoa (if previous failure of fertilization had occurred) for unexplained infertility.ICSI with husband's spermatozoa had similar FR to IVF with donor spermatozoa for patients with male factor. A significant proportion of the oocytes that failed to fertilize with conventional IVF eventually fertilized after ICSI.
Khamsi et al.[8] (2001)	Prospective controlled study	Non-male factor infertility.	35 patients	Per retrieved oocyte, ICSI resulted in better FR compared with conventional IVF. Per retrieved oocyte, ICSI also resulted in better formation of good-quality embryos at 48 hours after retrieval compared with conventional IVF.
Elizur et al.[9] (2004)	Retrospective study	Couples with mild oligoteratoasthenozoosp ermia and in couples with normal sperm and previous low fertilization rates (<30%) in conventional IVF.	69 couples	FR after IVF were significantly lower compared with those after ICSI, when male factor was present, but in the other group FR were not significantly different. The numbers of rapid-cleaving embryos at 2 days after fertilization were similar in both groups. Three days after fertilization, however, there were significantly fewer rapid-cleaving embryos after ICSI in the mild OTA group compared with aft er conventional IVF. There were no couples with complete fertilization failure in the normal sperm group, either after conventional IVF or after ICSI. In the mild OTA group, however, three couples had complete fertilization failure after conventional IVF, vs. none after ICSI.
Kim et al.[10] (2007)	Retrospective study	The study compared patient's characteristics, cycle, and outcomes.	696 consecutive assisted reproductive technology cycles	Patient characteristics were similar between the two groups. More oocytes were fertilized per cycle for the IVF group. Fertilization failure, PR, and live birth rates did not differ between IVF and ICSI. Using logistic regressions, having had previous ART was found to be positively associated with ICSI. Treatment choice of ICSI was not associated with better fertilization, pregnancy, or live birth rates.
Gozlan et al.[11] (2007)	Retrospective study	Stimulated or spontaneous IVF cycles resulted in single oocyte retrieval.	311 patients (425 cycles)	In patients <39 years old with favorable semen quality, ICSI and standard insemination produced similar FR and PRs. Conversely, in cases with apparent lower semen quality, ICSI gave a significantly higher fertilization rate (85.4% vs. 44.2%) but no significant difference in PRs. In patients >39 years old and with favorable semen quality, ICSI and standard insemination produced similar FR and PRs. The ICSI for lower semen quality produced both higher FRs and PRs.
Borini et al.[12] (2009)	Retrospective study	Each recipient received three to five eggs	601 women (671 cycles) donated their excess oocytes to 694 patients (1606 cycles).	The results showed that, when comparing donor versus recipients and among these, IVF vs. ICSI, no significant differences were found in terms of PR,IR and miscarriage rates.
Ou et al.[13] (2010)	Case-control study.	Couples had 1, 2 or 3 oocytes retrieved at PU	211 couples (243 cycles)	The FR was significantly higher after ICSI than after standard IVF. The cycle cancellation and complete fertilization failure rates were comparable between the 2 groups. The clinical PR per transfer, IR, and live birth rate per transfer had a favorable trend in the ICSI group, but no statistically significant differences.

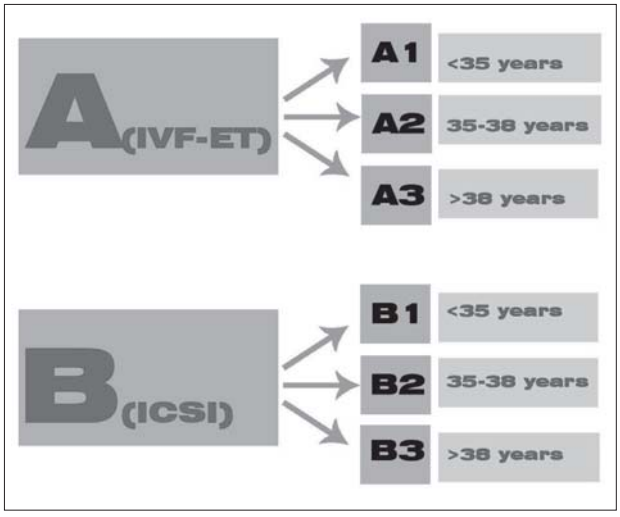

Fig. 1 - Design of the study.

Patients were excluded from the study if severe male factor was present.
The male partner submitted a semen sample for sperm analysis. Both male and female partners signed an informed consent form.
Ovarian stimulation was performed by a regimen including GnRH antagonists combined with rFSH treatment. The age of patients included in the study ranged from 27 and 47 years (mean age 38,4 yrs).
Cycles were divided into two groups based on the technique used: Group A was constituted by 45 FIVET cycles (43 patients, mean age 39,3 yrs), while Group B contained 38 ICSI cycles (35 patients, mean age 37,3 yrs), and results of the two group were compared. Some patients underwent both procedures, in different cycles. Group A and Group B were furthermore divided in 3 subgroups based on the age of patients (<35 yrs, 35-38 yrs, >38 yrs), whose results were also compared.
Finally, we analyzed and compared results of the group of patients in whom 1 oocyte was retrieved at pick up.

Laboratory procedure

Treatment protocol

Controlled ovarian stimulation was carried out with 150 to 450 UI/day, according to basal FSH levels and age, of recombinant FSH (Gonal F®, Serono, Italy). All patients were administered cetrorelix (Cetrotide®, Serono, Italy), a GnRH antagonist, according to a personalized regimen, i.e. when the lead follicle reached 14 mm in diameter, to prevent premature ovulation. Recombinant hCG (Ovitrelle®, Serono, Italy) was administered when at least 1-2 follicles reached a mean diameter of 18 mm. After approximately 36 hours, transvaginal follicular aspiration was performed for oocyte retrieval.
Progesterone supplementation was administered with 90 mg (Crinone; Merck Serono, Italy) via the vaginal route every day, plus 341 mg i.m. each 3 days (Lentogest; IBSA Farmaceutici Italia S.r.l.), starting on the day of embryo transfer.

Semen preparation

At the time of fertilization, sperm concentration and percent motility were used to assess fertilization potential of a given semen sample. Volume, sperm count, forward motility and morphology were considered according to the World Health Organization (WHO) criteria.
All semen samples were collected following masturbation after 3-4 days of abstinence and were allowed to liquefy for at least 20 min at 37°C before processing.
Sperm not meeting the defined threshold was classified as subfertile sperm, possessing questionable fertilization potential and patients were consequently excluded from the study.

Oocyte preparation

Oocytes were retrieved by vaginal ultrasound probe and incubated in oocyte culture medium (Sydney IVF Oocyte Wash Buffer; Cook Ireland Ltd, Limerick, Ireland).
Fertilization technique was chosen based on the clinical history of patients and reproductive outcomes in previous ART cycles. In cases where we choose to perform ICSI, we hypothesized that the impairment in fertilization was the cause of the past failures in previous ART cycles.
4-5 h after oocyte retrieval, in cases where IVF was performed, each oocyte was inseminated with 200,000-300,000 motile washed spermatozoa, and before proceeding to ICSI, the cumulus cells were removed from the oocytes after 3-4 h of incubation in hyaluronidase (type VIII; Sigma, USA) at a concentration of 80 IU/ml for approximately 15-20 s.
Thus, oocytes were transferred to fresh medium and the corona cells were removed by softly pipetting in and out. The oocytes were subsequently controlled under an inverted microscope at x100 to check the maturation stage, based on the presence of the first polar body.
ICSI was accomplished as previously described by Artini *et al.*, 1998 (14).
Fertilization was confirmed by the observation of two

pronuclei about 14-18 h after fertilization technique. All the fertilized oocytes were transferred into a fresh cleavage medium and cultured until transfer. On day 2, at 46-48 h post-insemination, the embryos were evaluated for cell number and rate of fragmentation and consequently graded as I-IV (best–worst) (15).

Results

IVF-ET vs ICSI (A vs B n=83)
In regard to fertilization rate, there were no differences between the Group A (79%) and Group B (68%). On the other hand, taking into consideration good quality embryo rate, Group A obtained 65%, versus 72% of Group B. For what concerns pregnancy rate, IVF-ET was found to be more advantageous (biochemical PR 13 vs 11%; clinical PR 11% vs 5%). (Table 2)

Age <35 years (A1 vs B1, n=18)
When fertilization rates using fertile sperm were compared between ICSI and conventional IVF in this subgroup of patients, results were similar in both groups (87% vs 73%). (Table III)
For what concerns good quality embryo rate, ICSI group <35 yrs (B1) obtained a better result (A1 57% vs B1 69%). Furthermore, biochemical and clinical PRs using fertile sperm were superior after IVF-ET compared to ICSI (40% vs 8% and 40% vs 8%, respectively).

Age 35-38 (A2 vs B2, n=22)
In this subgroup of patients, we found that ICSI did not significantly improve the parameters evaluated (Table 3).

Age >38 (A3 vs B3, n=43)
In this subgroup of patients, we found that IVF-ET improved fertilization rates. In fact, Group A3 obtained a better result (82% vs 60%). For what concerns good quality embryo rate, ICSI group >38 yrs (B3) was shown to be more effective (A3 63% vs B3 75%). When biochemical PRs were compared between ICSI and conventional IVF, there were no differences between the two groups, on the contrary clinical pregnancy rate was better in IVF-ET group (7% vs 0%). (Table 3)

Comparison of IVF-ET and ICSI in patients with single oocyte retrieval

From the analysis of this subgroup of patients, we highlighted no significant differences for all the parameters tested. (Table 4)

TABLE 2 - OUTCOMES OF IVF-ET AND ICSI.

	Group A IVF-ET n=45 (54%)	Group B ICSI n=38 (46%)
Biochemical pregnancy rate	6 (13%)	4 (11%)
Clinical pregnancy rate	5 (11%)	2 (5%)
Miscarriage rate	2 (40%)	1 (50%)
Number of oocytes retrive	74	60
Fertilization rate	59 (79%)	41 (68%)
Embrio cleavage rate	57 (96%)	39 (95%)
Good-quality embrio rate	37 (65%)	28 (72%)

TABLE 4 - COMPARISON OF IVF-ET AND ICSI IN PATIENTS WITH SINGLE OOCYTE RETRIEVAL.

	IVF-ET n=16	ICSI n=16
Biochemical pregnancy rate	1 (6%)	2 (13%)
Clinical pregnancy rate	1 (6%)	1 (5%)
Miscarriage rate	0 (0%)	1 (100%)
Number of oocytes retrieved	16	16
Fertilization rate	13 (81%)	13 (81%)
Embrio cleavage rate	13 (100%)	13 (100%)
Good-quality embrio rate	7 (53%)	9 (69%)

TABLE 3 - OUTCOMES OF IVF-ET AND ICSI ORGANIZED ON THE BASIS OF AGE.

	Age <35		Age 35-38		Age <38	
	Group A1 IVF-EN n=5 (6%)	Group B1 ICSI n=13 (16%)	Group A2 IVF-EN n=11 (13%)	Group B2 ICSI n=11 (13%)	Group A3 IVF-EN n=29 (35%)	Group B3 ICSI n=14 (17%)
Biochemical pregnancy rate	2 (40%)	1 (8%)	2 (18%)	2 (18%)	2 (7%)	1 (7%)
Clinical pregnancy rate	2 (40%)	1 (8%)	1 (9%)	1 (9%)	2 (7%)	0 (0%)
Miscarriage rate	1 (50%)	1 (100%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)
Number of oocytes retrive	8	22	20	18	46	20
Fertilization rate	7 (87%)	16 (73%)	14 (70%)	13 (72%)	38 (82%)	12 (60%)
Embrio cleavage rate	6 (86%)	15 (94%)	13 (92%)	12 (92%)	38 (100%)	12 (100%)
Good-quality embrio rate	4 (57%)	11 (69%)	9 (64%)	8 (62%)	24 (63%)	9 (75%)

Discussion

When dealing with male factor infertility, it is well known that use of ICSI is recommended. However, when seminal parameters are normal, is still a debated dilemma if the ICSI technique brings any special benefit with respect to IVF. Although some studies in literature show that ICSI is advantageous for poor responder patients, others failed to demonstrate any superiority of ICSI on conventional IVF.
Moreover, our results confirmed the studies that questioned the advantages of ICSI over conventional IVF in patients with non–male factor infertility as well as patients with poor ovarian response.

Conclusions

Our results allow us to affirm that ICSI is not always the gold standard in the management of poor responder patients, especially those in whom only one or two oocytes are retrieved at oocytes pick up. As a consequence, probably performing ICSI in all these patients is not useful, and surely it is more expensive and time consuming.
In conclusion, when choosing the ART technique it is better to consider the single patient and all her clinical characteristics, such as age, and her response to ovarian hyperstimulation in the previous cycles.
ICSI is recommended as the technique of choice only in case of male factor infertility, while in other cases is still object of controversy if it is better to perform ICSI or IVF-ET.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Postmenopausal period in acromegalic women

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Acromegaly is rare severe neuroendocrine disorder due to chronic excess of growth hormone (GH) and insulin-like growth factor type 1 (IGF-1). The principal cause of the increased GH release is usually a benign pituitary tumor – somatotropinoma, in 75% of cases pituitary tumors are more than 10 mm in size and with extrasellar extension (macroadenoma).

Acromegaly commonly occurs in middle-aged men and women, male to female ratio is 1:2. The prevalence of the disease is about 6 of every 100,000 adults, and the incidence is 3-5 new cases per million per year. The clinical features associated with acromegaly include the effects of GH over-production and – in instance of macroadenoma – effects of tumor compressing and injuring the normal pituitary gland, optic nerves and optic chiasm.

Active acromegaly results in marked bony and soft tissue changes including an altered facial appearance (frontal bossing, prognathism), enlargement of the hands and feet, sleep apnea, and carpal tunnel syndrome, arthritis, excessive sweating, besides tiredness, fatigue, depression, hypertension, impotence, loss of libido, interrupted menstrual cycle and some other clinical symptoms.

In the Moscow Region 140 patients with acromegaly are included in special Acromegalic Registry. According to this Acro-Registry, among acromegalic patients there are 111 (79%) women, including 60 (43%) women in a postmenopause. Thus, up to 43% of patients with acromegaly are postmenopausal women. However, it is not enough data on this topic.

We examined 27 postmenopausal women 50-73 years old (median 57) with acromegaly. Median duration of acromegaly 13.3 years; active acromegaly in 19 (70.3%) patients, remission in 8 (29.7%) cases. Macroadenomas had 74% of patients, microadenomas – 26%. At patients with active acromegaly (n=19) median of basal GH was 8.6 [4.3; 19.9] mMe/l, median of IGF-1 was 404 [317; 819] ng/ml (reference range 94-269).

Sixteen women had natural menopause, median menopause age 50 [47; 51]. Uterine myomas were observed in 10 (37%) patients, including 4 (14.8%) women with *de novo* myomas. In most cases myomas were asymptomatic, of small size, with low blood flow in the uterine vessels and the vessels feeding the myoma node; in 1 case it was submucosal myoma that required surgery treatment. Myomas were associated with other pathological conditions: adenomyosis (n=3), endometrial polyps (n=3) and ovarian teratomas (n=3). Ovarian cystomas were found in 3 other patients.

Eleven women (40.7%) had been operated concerning a progressing uterine myoma before the study, median surgical menopause age 47 [43; 50] years old, there was no significant difference compared to median age of natural menopause (p=0.05). In 6 (22.2%) cases myomectomy was performed before the appearance of other typical symptoms of acromegaly. Age of surgical menopause at these women varied from 38 to 48 y. Fast growth of myoma that require surgical treatment could be considered as one of the first symptoms of acromegaly, and determination of GH & IGF-1 levels can be recommended in these cases.

Interestingly, 4 (14.8%) patients (2 with natural and 2 with surgical menopause) had hypogonadotropic hypogonadism: LH ranged from 1.1 to 5.1 U/l, FSH – from 4.1 to 15.5 U/l, PRL 43-170 mMe/l. All patients had macroadenoma and were operated on pituitary (in 1 case in combination with radiotherapy). Age of menopause was 38, 45, 46 and 51 years.

In other patients (n=21), an adequate increase of gonadotropin levels were observed: median of FSH was 47.2 [37; 66.3] mIU/ml, median of LH was 15.8 [11.5; 22.3] mIU/ml.

Only 1 woman from our cohort with *de novo* acromegaly had hyperprolactinemia 1115 mMe/l. She has natural menopause at 48 y.o. and her gonadotropin levels were not suppressed: LH 11.5 U/l, FSH 39.4 U/l. We considered that probably hyperprolactinemia was a consequence to mass effect of macroadenoma.

Median score according to Greene Climacteric Scale was 23. Only few patients had moderate or severe symptoms like panic attacks (Q5, 33.3% of patients), difficulty in concentrating (Q6, 14.8%), loss of interest in most things (Q8, 29.6%), feeling unhappy or depressed (Q9, 3.7%), crying spell (Q10, 14.8%), irritability (Q11, 18.5%), feeling dizzy or fain (Q12, 18.5%), parts of body feel numb or tingling (Q14, 7.4%) hot flashes (Q19, 14.8%). Approximately half of the patients complained on heart beating quickly or strongly (Q1, 44.4%), feeling tense or nervous (Q2, 51.8%), difficulty in sleeping (Q3, 51.8%), pressure or tightness in head or body (Q13, 48.1%), loss of feeling in hands or feet (Q17, 40.7%), breathing difficulties (Q18, 40.7%), sweating at night (Q20, 40.7%), loss of interest in sex (Q21, 51.8% of patients). The majority of patients suffered from moderate or severe headache (Q15, 70.3%), feeling tired or lacking in energy (Q7, 70.3%), muscle and joint pain (Q16, 70.6%). Nevertheless, symptoms of active acromegaly are similar to some postmenopausal symptoms and may contribute to the high Greene score.

Urogenital disorders (stress incontinence) that required further treatment were detected in 14 (51.8%) patients. The frequency of urogenital disorders was higher among patients with surgical menopause compared to women with natural menopause (9 from 11 vs 5 from 16 patients respectively, p=0.02)

In conclusion, various gynecological disorders were detected in all postmenopausal women with acromegaly. Median age of menopause did not differ from general population, however, prevalence of uterine myomas (77.8%) was much higher in the acromegalic women than in the population (up to 40% in Russian population). Fast growth of myoma that require surgical treatment could be considered as one of the first symptoms of acromegaly, and determination of GH & IGF-1 levels can be recommended in these cases.

According to our study, some climacteric symptoms and clinical signs of acromegaly might be similar (for example, headache, feeling tired or lacking in energy muscle and joint pain, heart beating quickly or strongly, sweating at night, breathing difficulties) so it is difficult to differentiate them. Probably, due to this reason Greene Climacteric Scale is not appropriate for acromegalic postmenopausal women. Among climacteric symptoms urogenital disorders were very common (51.8%) especially at women with surgical menopause.

The frequency of hypogonadotropic hypogonadism was 14.8% at our cohort, and the main cause was operation on the pituitary macroadenoma. Hyperprolactinemia is not common among postmenopausal patients with acromegaly.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Endothelial function and glucose metabolism in overweight and obese nondiabetic women: a possible role of L-Arginine/Nitric Oxide pathway

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Objective

We have previously demonstrated that with an acute L-Arginine (L-Arg) infusion we were able to reveal a Nitric-Oxide Syntase (NOS) impairment in pre-eclamptic (PE) women, as demonstrated by significantly lower L-Citrulline conversion than in Controls (1). Moreover, an acute L-Arg infusion induced a vasodilation in placenta-related foetal growth restriction (2). On the other hand, L-Arg/NO pathway is also involved in glucose metabolism. In particular, Insulin exert an important biological effect on adipose tissue, skeletal tissue and vascular tissue, through different intracellular kinase signaling including PI3-kinase activation, glucose transport, by Glut 4 and Glut 1 activation, and NO production by Akt activation (3,4). Near this physiological metabolic pathway, in several situation like during Insulin-Resistance (IR), Insulin induce extracellular signal-regulated kinase (ERk1/ERk2) and, as a consequence, MAPk (Mitogen-activated protein kinase) that lead to Endothelin-1 production and atherosclerosis onset, while, on the other hand, there is a deficiency of intracellular signaling transmission.
Insulin resistance and resultant hyperinsulinemia are physiological characteristics of pregnancy, expecially in third trimester. In obese women this situation is exasperated and may be associated with the endothelial dysfunction. Both glucose metabolism and vascular activity are modulated by L-Arg/NO pathway: endothelial NO production and Insulin sensitivity are positively related in healthy people (5). Unfortunately, in obese people, such system seems blunted. Considering that the prevalence of obesity is increasing in the general population and top BMI (Body Mass Index) categories in pregnant women are also increasing, we aim at evaluating L-Arg-NO regulatory system in different BMI categories during pregnancy.

Methods

Eight normal weight (BMI 18.3-24.9 Kg/m^2) and 14 overweight/obese women (BMI 26.3-45.2 Kg/m^2) underwent twice during pregnancy (at 9-12th and at 24-27th weeks) to L-Arginine infusion (20g in 500 ml physiological solution, in 3 hours). Serum assay for glucose, insulin and nitrite/nitrate (NOx) levels were fasting and hourly performed. Serum NOx concentration was obtained by reduction of nitrate to nitrite with cadmium. The essay was performed with colorimetric method based on Griess reaction. The results are expressed in µM/L. The quantitative determination of plasma insulin was obtained by ELISA method. Concentrations are expressed in µIU/ml.
The ultrasound assessment of endothelial-dependent flow mediated vasodilation (FMD) of the brachial artery was also performed, measured as the percent change of brachial artery diameter in response to increased blood flow shere stress (6).
Data obtained were recorded on a database and analyzed through SPSS® Statistics version 19. Comparisons between groups were performed using Student's t test for independent samples. For the analysis between the same group we used Wilcoxon's test for non-parametric models. For comparisons, NOx production was evaluated as Area Under the Curve. All values are expressed as mean ± standard deviation. We consider p value less than 0.05 as threshold for statistical significance.

Results

At 1st trimester L-Arg infusion induce a glucose decline in both groups. In overweight/obese women such reduction is more evident (p=0.04). At 2nd trimester the reduction is present only in obese/overweight women (p=0.03). Fasting insulin levels were significantly higher in overweight/obese than lean group: 19.1±18.1 vs 7.9±5.1 µUI/ml at 1st trimester (p=0.05) and 21.3±8.0 vs 12.1±5.1 µUI/ml in 2nd trimester (p=0.03). At 1st trimester, L-Arginine significantly reduced insulin levels in both groups. NOx levels were similar in both groups at 1st trimester, but the difference between groups became significant at 2nd trimester with NOx values significantly higher in normal weight women than in overweight/obese women after 120 (p=0.03) and 180 minutes (p=0.05). FMD of brachial artery at 2nd trimester was significantly reduced in overweight/obese (16±13%) vs lean (32±6%, p=0.03) women.

Conclusions

Our study showed that overweight and obesity actually cause metabolic alteration from the earliest stages of pregnancy, as demonstrated by higher glucose and insulin levels at 1st trimester in women with BMI >25 kg/m^2. This agrees with several studies that correlate obesity ad insulin resistance. NO production in response to L-Arg administration is overall similar between groups at 1st trimester. This agrees with the response in terms of endothelium-dependent vasodilation assessed by FMD, which is preserved in both groups (Table 1).

TABLE 1 - 1ST TRIMESTER NOx LEVELS AND ENDOTHELIAL-DEPENDENT VASODILATION.

	Normal-weight	Overweight/obese	*p* value
NOx (AUC)	7953.0 ± 6711.2	7779.9 ± 5608.3	0.953
FMD (%)	67.4 ± 32.9	24.7 ± 16.9	0.078

At 2nd trimester in normal-weight patients we observe the physiological increase in insulin levels, but overweight/obese women maintain higher glucose and insulin levels. It is evident the poor response of overweight/obese women in terms of NO production, which is, instead, increased in normal-weight women. This impacts on vascular function: at FMD endothelium-dependent vasodilation is significantly reduced in overweight/obese patients (Table 2).

TABLE 2 - 2ST TRIMESTER NOx LEVELS AND ENDOTHELIAL-DEPENDENT VASODILATION.

	Normal-weight	Overweight/obese	*p* value
NOx (AUC)	8194.0 ± 2906.8	5576.7 ± 3794.2	0.045
FMD (%)	32.2 ± 6.7	16.8 ± 13.3	0.033

Our data suggest an alteration of L-arg-NO system at 2nd trimester, shown as a reduced NO production associated with a decrease in endothelium-dependent vasodilation in overweight/obese women.
In conclusion, L-Arginine-NO pathway seems impaired in overweight/obese women at 2nd trimester and it is associated with a reduction in FMD. These findings may explain vascular and metabolic dysfunction in women with BMI >25 Kg/m^2. If chronic L-Arg supplementation could be helpful to obese women for glucose utilization remain to be discovered.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The low risk for fracture in postmenopausal women. Quantitative ultrasound evaluation in 143 patients

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Introduction

Except for the golden standard DXA (Dual energy X-Ray Absorptiometry), the quantitative ultrasound (QUS) is a practical device to evaluate the fracture risk in women after menopause. The best site is the heel and the most accepted results are based on GE Lunar Achilles studies. (1) The Stiffness Index (SI) indicates the risk fracture and it represents the combination of BUA (Broadband Ultrasound Attenuation) and SOS (Speed of Sound) parameters. (2) As suggested previously in literature, the similar parameters as Z or T-Score do not apply in QUS, as in DXA. (3) In patients with no fragility fractures and high SI (over 80 Units) DXA is not necessary, neither therapy for osteoporosis as they are considered as having the lowest risk for osteoporotic fractures. Generally, the data from literature suggests that the combined results of DXA and QUS identify best at risk patients but there are some specific segments of the population to whom DXA (as the most expansive of the two devices and more difficult to perform) is not necessary. (4)

Aim

We compare the DXA and other parameters of the low fracture risk group (baseed on QUS results) to non-low risk patients.

Subjects and methods

All the patients were women in least 6-12 months of secondary amenorrhea (postmenopausal) who were antiresorbtives-free. They were all evaluated by lumbar and hip GE Lunar DXA and Heel GE Achilles Quantitative Ultrasound. The study was conducted for 2 years (2010-2011) in CI Parhon National Institute of Endocrinology from Bucharest, Romania. The Body Mass Index (BMI) was also registered. We analyzed the bone turn over markers like formation markers (serum osteocalcin) and resorption markers (serum alkaline phosphatase and CrossLaps). Moreover, the bone status hormones were performed serum parathormon (iPTH) and serum 25 OH-vitamin D. We considered as low risk for fracture 143 patients to whom the SI was ≥80 U. The control group included 200 women with the same inclusion and exclusion criteria but with heel SI <80U. The statistical analyze included student ttest, statistically significant being $p<0.05$.

Results

The studied group included the women SI between 80 and 143U. The av. age for the low risk group was 55.99±7.41 year, as for non-low risk group was 57.23±9.28 years. As expected, the low risk patients are younger but not with a strong significance ($p=0.06$). The av. BMI for the low/ non-low fracture risk groups was: 29.93±5.46, respective 27.92±5.94 g/cm2. The BMI increases as the fracture risk decreases, with statistical significance ($p=0$). The av. fat mass percent as revealed by Body Mass Analyzer was 37.64±18.55% versus 39.2±10.88% in control groups. This was not statistically significant ($p=0.4$). Also, it is important to mention the fact that the fat mass influences the QUS measurements as it interfere

with the wave. (5) The av. serum Alkaline Phosphatase was within the normal range (60-210U/l) and did not differ from the groups: 77.89±27.49U/l versus 77.63±27.72U/l in low risk groups (p=0.91). The serum CrossLaps were lower in low risk women 0.43±0.25ng/ml versus 0.52±0.3ng/ml (p=0.01), with normal values between 0.2 and 0.704 ng/ml. The av. osteocalcin was lower in low risk patients: 21.51±0.25 ng/ml versus 24.46±12.97 ng/ml, also with av. values within the normal range of 14 up to 46 ng/ml (p=0.04). These last two parameters were statistically significant. The iPTH is not statistically significant between the groups (46.75±24.01 pg/ml in low risk patients, and 50.34±36.67pg/ml in control group, with normal ranges of 15 to 65 pg/ml, and p=0.32). Similar results were found in average levels of 25 OH-vitamin D: 13.66±7.14ng/ml versus 13.29±7.05ng/ml in non-low risk groups, with deficiency of vitamin D based on average values.

The lumbar BMD was statistically significant higher in low risk fracture group, as 1.1±0.19 g/cm^2 versus 0.98±0.17 g/cm^2 (p=0), as well as femoral neck BMD 0.94±0.15 g/cm^2 versus 0.84±0.11 g/cm^2 (p=0), and hip BMD 1.05±0.15 versus 0.92±0.11 g/cm^2 (p=0).

Discussions

The changes in bone turn over markers, as well as the fat tissue clues are important in evaluation of the bone health. We realized an uni-variable analyze between the groups of patients who were at low risk of fragility fractures and a control group, meaning women with high and intermediate risk for fracture. The low risk group is important to be identified especially for economical reasons because no DXA is necessary for them, only follow up by heel QUS. The studied group differs statistically significant by BMI, but not by fat mass percent. They tend to be younger. The serum osteocalcin and CrossLaps are higher, while the iPTH and 25 OH-vitamin D are not different between the groups. Good results were found in BMD-DXA analyze, meaning that the BMD is lower in patients with increased risk for fracture based on SI QUS.

Conclusions

We consider that based on these results in low risk for fragility fractures women, the bone markers and body mass index indicate a difference to the higher risk population, while the two sites central DXA results confirm the differences between the two groups.

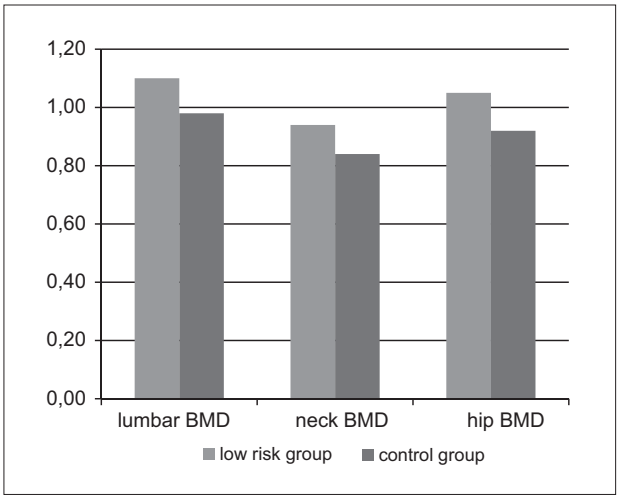

Fig. 1 - The BMD values (g/cm^2) for lumbar spine, femoral neck and hip in low risk for fracture, respective control groups.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Etiological treatments for couple's infertility: a single-center retrospective analysis

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Introduction

Infertility affects 13% to 15% of the couples worldwide (1). A male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40% (2). If a male infertility factor is present, it is almost always defined by an abnormal semen analysis. However, since WHO references were adopted, it has become evident that a basic semen analysis is insufficient to determine the fertility status of the male (3).
Subclinical hypothyroidism is more frequent in women with ovulatory disorders than in women with other causes of infertility (4) and can be considered as an infertility factor by itself (5). Levothyroxine (LT4) therapy can reverse thyroid disfunction and may improve fertility.
Therefore, tubal factor infertility accounts for approximately 25-35% of cases of female infertility. Identifiable causes of tubal infertility are postinfectious tubal damage, post-surgical adhesion formation, and endometriosis-related adhesions (7).
We report a large retrospective study in a population of infertile couples who conceived after ethiological therapies and provide informations regarding the relationship between semen parameters and spontaneous conception, the association between ovarian disfunction and thyroid function and the role of a minimally invasive approach in the diagnosis and treatment of tubo-peritoneal infertility.

Materials and methods

3807 infertile couples were evaluated at our infertility clinic between January 2004 and December 2010. Each couple underwent evaluation by gynaecologists, andrologists and endocrinologists. Physical examination, hormonal assessment of ovulatory function, screening for cervical and vaginal infections, transvaginal sonogram, evaluation of tubal patency and genetic assessment (if indicated) were carried out in the female patients.
The male patients underwent physical examination, genetic assessment and evaluation for endocrine factors, non-endocrine testicular dysfunctions and accessory gland infections. Standard semen analysis was performed periodically, according to WHO guidelines (8).
Etiological treatments were performed in presence of identified causes of infertility. ARTs were not performed, according to our ethical guidelines. All couples expressed informed consent to this protocol.
592 couples obtained a spontaneous pregnancy. Clinical and laboratory data, including standard semen analysis and female thyroid function, were retrospectively evaluated. Pregnancy rate was calculated for 152 patients who underwent, for tuboperitoneal infertility, diagnostic or operative laparoscopy and hysteroscopy.

Results

Normal semen analysis was present only in 35% of patients; 65% subfertile patients showed alterations in at least one seminal parameter. A sperm count lower than WHO reference values, isolated or associated with motility or morphology alterations, was present in 26% of the total pregnancies. Isolated asthenospermia was the most frequent semen abnormality and was present in 27% of the total pregnancies. 9% of the to-

tal pregnancies were obtained by men with oligoasthenoteratospermia.
65 women, without other infertility causes than thyroid disfunction, began LT4 therapy no more than 6 months before the conception. When stratified according to TSH values, 32 patients had a TSH lower then 2.8 mcUI/ml while in 33 was described a sublinical hypothyroidsm. In the group of patients with TSH<2.8 mcUI/ml, 15 had an autoimmune thyroiditis, 6 had a positive TRH test and in the remaining 110 was reported an ultrasound pattern of thyroid nodes.
Of the 152 patients who underwent diagnostic or operative laparoscopy and hysteroscopy for tuboperitoneal infertility, 61 obtained a pregnancy (40%). Twenty-three pregnancies resulted in miscarriage, 2 in tubal pregnancy and one patient aborted after a diagnosis of Down syndrome. 32% of the patients achieved a term pregnancy.

Discussion

We report 592 pregnancies occurred between 2004 and 2010 among 3807 infertile couples evaluated at our institute. In the infertile couples, we applied standard protocol in terms of investigation and therapeutical management of infertile couples, performed in both partners in a sequential and parallel way. This was achieved by a unified clinical management of the couple, which include gynecologists, endocrinologists and andrologists, with specific interest in the field of human reproduction.
Our data support the possibility of spontaneous conception with semen parameters below the WHO references, as documented by the 65% of pregnancies that occurred in our center, and call into question the predictive value of WHO reference values, which are not always predictive of fertility. In order to improve the clinical value of seminal parameters, WHO revised its laboratory manual for the examination and processing of human semen (9), giving the lower reference limit for seminal parameters at the 5th percentile in a population of men of proven fertility, in agreement with our evidence of spontaneous conception by men with reduced seminal parameters.
Our data evidence therefore that subclinical hypothyroidism is a cause itself of female infertility. In the couples with TSH values higher than 2.8 µUI/ml, the LT4 treatment achieved pregnancy. In women with idiopathic infertility the presence of TSH value in the upper limit of the normal laboratory references, with associated thyroid alterations at laboratory or ultrasound evaluation, could represent also an indication for treatment with levothyroxine in hypofertility.
The diagnostic/therapeutic mini-invasive approach for tuboperitoneal infertility allowed to obtain pregnancy naturally with a pregnancy rate of 40% and 32% for term pregnancy. When efficacious, this approach allows additional spontaneous conceptions without renewed therapy.
Finally, our experience confirm the importance of etiological therapies for infertility. As a consequence ART techniques should be considered only after a sufficient time of etiological therapies, also according to ethical criteria of distributive justice, fairness and prioritization (9).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Mineral bone mass as a risk factor for temporomandibular disorders

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Introduction

Osteoporosis is the result of the bone remodeling imbalance, which depends on the action of Rank-RankL system, OPG, M-CSF, cytokines and hormones. Hypoestrogenism acts on bone loss, increasing cytokines levels, osteoclasts differentiation and activation, beyond promoting osteoblasts and osteocytes apoptosis (1).
Temporomandibular disorder (TMD) is more prevalent in women and it is believed that sex hormones are part of its etiology, because it begins at puberty, with peaks during the reproductive period and decreasing after menopause, also cause different responses on temporomandibular joint (TMJ) among the genera (2,3).
Hypoestrogenism leads to cartilage thickening, condyle flattening and increase of eroded surfaces (4), otherwise, high levels of estrogen causes the opposite (5).
Essential remodeling elements (OPG and RankL) are present in the TMJ and the greatest OPG expression occurs in the presence of estrogen in less committed joints (6,7). The level of RankL decreases with estrogen, not related to the TMJ degeneration (8).
The jaw restraint (immobilization) results in TMJ osteoporotic characteristics (9).
Given the sexual hormones involvement during the osteoporosis and doubts about their influence over the TMD, we analyzed whether these two diseases predominantly female were related, whereas bone loss might be a possible risk factor for articular TMD.

Methodology

From 2008 to 2011, 1291 women were evaluated by a single examiner.
Inclusion criteria: age between 48 and 70 years old, without hormone replacement therapy for at least 1 year or any medication that could interfere with bone metabolism and current routine tests (hemogram and bone densitometry).
Exclusion criteria: diabetes, fibromyalgia, thyroid disorders, rheumatoid arthritis, osteoarthrosis, autoimmune diseases, Lyme disease, dyslipidaemia, disorders of calcium metabolism and parathyroid hormone levels.
Densitometry exams were done on a QDR-4500A densitometer for analysis of the lumbar vertebrae and femoral neck. The values of T score grouped the patients, based on established criteria in 1994 by the WHO. Articular TMD diagnoses were obtained with the RDC/TMD, classifying patients with up to 5 diagnoses within 3 groups: muscle disordersI) and Group III (arthralgia, osteoarthritis or osteoarthrosis). In this work, only articular diagnoses were considered, namely those from Groups II and III.
Catmaker System (2004) calculated bone mass risk offered to the articular TMD, structuring the "PICO": Patient (women between 48 and 70 years), Intervention (osteopenia/osteoporosis), Comparison (normal bone) and Outcome (articular TMD). It was assumed significance level of 0.05. The sample size calculation was based on increased relative risk, and for a 80% power, only 40 osteopenic and 30 osteoporotic women could've been considered.

Results and discussion

Osteoporotic women (n=34) had higher mean age (59.1±5.7), while osteopenic (n=46) had an average of 57.5 (±4.4) years old. The normal bone mass group

(n=20) had an average age of 56.3 (±5.1). From all obtained diagnoses, 18% corresponded to Group I, not considered for analysis. Were identified 68% articular diagnostic as a likely consequence of systemic bone loss, since most of them were present in osteoporotic women (25%). These were compared to the absence of diagnoses (14%) present in most women with normal bone mass (Table 1). The lack of estrogen increases the TMJ cartilage thickness, flattening the articular surfaces (degenerative diseases characteristic) and reduce bone volume and osteoid (4). Estrogen excess reduces chondroblasts number and maturation, thinning the cartilage and decreasing proteoglycan content, making TMJ less resistant to disorders (5). As the condyle mitotic compartment is at a subarticular layer, hypoestrogenism activate remodeling units located there, estimulating resorption. Thus, estrogen at physiological levels is critical to TMJ health. As evaluated women were over a declining estrogen period, hypoestrogenism bone effects reflected on their joints, given that, from the total joint diagnoses, 25% were osteoporosis and 33% osteopenia. Of these, 16% were in severe osteopenia, ie, closer to osteoporosis than normal bone. All these aspects confirm the existence of estrogen receptors at TMJ (4,5). Hypoestrogenism promotes the expression of cytokines and osteoclast precursors, enhancing bone resorption (1). These elements are also found in inflamed TMJ, where cells differentiation into osteoclasts stimulates bone degeneration (8).
The risk posed by osteoporosis to articular TMD was higher than osteopenia, respectively 1.39 ($CI^{95\%}$ 1.23 to 1.55) and 1.33 ($CI^{95\%}$ 1.20 to 1.46), increased by 33% to osteopenia and 39% to osteoporosis (ARR of 0.33 and 0.39). The mandibular function protected TMJ from bone loss in animals (9), but did not spare bone loss risk at women assessed here, since all were doing this function properly. It is known that the resistive movement produces bone, different from the usual in terms of impact, but in the case of masticatory system, it would be a parafunction.
In equilibrium, OPG and RankL-Rank system ensure TMJ bone metabolism. However, there is less OPG in joints with degenerative diseases, ie, the cartilage degradation is greater due to the lower OPG concentration (6,7), showing the possibility of therapeutic benefits of its use at TMD by bone destruction. Despite the subtle expression of RankL in dysfunctional joints (8), its presence confirms the existence of a cellular source for osteoclastogenesis, that would be more related to cartilage degradation than bone destruction itself.
We conclude that the TMJ component also suffers hypoestrogenism negative consequences, especially in osteoporotic women.

TABLE 1 - TOTAL DIAGNOSTICS (TMD) X BONE.

Bone mass		TMD	No TMD
Normal		16 (9%)	9 (5%)
	Slight	23 (12%)	
Osteopenia	Moderate	10 (5%)	6 (3%)
	Severe	31 (16%)	5 (3%)
Osteoporosis	48 (25%)	6 (3%)	
	Total*	128 (68%)	26 (14%)

* maximum 4 articular diagnoses: 2 for each TMJ.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Estrogen receptor α and β cooperate with GPR30 in the regulation of estrogen-induced ERK activation in primary human endometrial stromal cells

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Introduction

Human endometrium is a hormone dependent tissue in which cells undergo a variety of adaptive reactions in response to the hormonal changes that occur during the menstrual cycle. These changes involve various signaling pathways, which regulate the behavior of endometrial cells such as proliferation, migration and apoptosis. The actions of 17-ß-estradiol can be mediated through the two classical estrogen receptors estrogen receptor α (ERα) and estrogen receptor ß (ERβ). They are ligand-activated nuclear transcription factors and mainly responsible for the genomic estrogen signaling (effects on gene expression). A third receptor emerged in the last decade to also mediate estrogen-induced effects, namely a seven transmembrane G-protein coupled receptor GPR30 (=GPER1, G-protein coupled estrogen receptor 1). This receptor is thought to mediate non-genomic estrogen effects in many tissues of the body by rapid modulation of cell signaling pathways eg. myometrium, brain, vasculature. It is also able to indirectly exert transcriptional activity (1). The role of GPR30 in terms of contributing to carcinogenesis in the breast or endometrium is now under intensive investigation (2). Therefore to explore the physiological role of this receptor in the reproductive system is very important to understand its ways of participating in hormonal signal transduction (3).

Aim

In this study, we examine the role of the estrogen receptors ERα, ERβ and G-protein coupled estrogen receptor (GPR30) in the regulation of the 17β estradiol (E2)-induced non-genomic MAPK/ERK activation in normal primary human endometrial stromal cells (hESC).

Materials and methods

hESC obtained from eutopic endometrium of women (n=10) undergoing laparoscopy and curretage during the proliferative phase of the menstrual cycle were used. The absence of endometrial disease was confirmed by additional histological examination. Before stimulation, the cells were cultured in serum free DMEM-F12 medium for 24 hrs and, when indicated, 12 hrs before stimulation they were pre-treated with either vehicle or specific inhibitory compounds. In the present study the following inhibitory compounds were used: 1 μM ER inhibitor ICI 182,780, 100 ng/ml specific G-protein inhibitor pertussis toxin (PT), 1 μM EGFR inhibitor AG1478, 150 nM Src tyrosine kinase inhibitor (Src-I), 5 μM specific Src family of tryrosine kinases inhibitor PP2 and 1 μM specific GPR30 inhibitor G15. The cells were stimulated with: either E2 (1 nM or 1 μM), or PPT, or 10 nM specific ER-β agonist DPN or with 1 μM or 1 nM GPR30 specific agonist G1 in the presence or absence of inhibitors for short periods of time (from 2 to 60 min) at 37°C. PCR and Western blot analyses for ER-α ERβ, GPR30 and Western blots for pERK, total ERK and α-tubulin were performed in control and treated hESC as well as after specific GPR30 and ERα-siRNA knockdown. Real time (Q)-PCR was performed 48 hours after the initial GPR30-knockdown in hESC without hormonal stimulation and mRNA-levels of GPR30, ERα and ERβ were determined.

Results and discussion

Maximal ERK activation was observed within ten min following either E2 or G1 (GPR30 non-steroidal agonist) administration, which was abolished by the specific inhibitors ICI 182,780 or G15, respectively. The inhibition of ERs with ICI 182,780 abolished GPR30-mediated ERK activation and the inhibition of GPR30 with G15 abolished the stimulatory effects of E2, respectively. We further show that G-proteins and Src kinase family members are required for efficient ERK activation in hESC. The specific EGFR phosphorylation inhibitor AG1478 blocked the E2- and G1-induced ERK activation in hESC.
Knockdown of either ERα or GPR30 confirmed the data obtained from antagonist treatments and showed that both receptors are involved in the regulation of MAPK/ERK signaling pathway activity after hormonal stimulation. In addition to the changes in ERK activation, GPR30 knockdown in hESC coursed significant reduction of c-jun expression levels as well as in the levels of pAkt seen after G1 stimulation. Long-term treatment of hESC with either E2 or G1 alone or in combination with a MAPK inhibitor (PD) showed E2-induced ERK activation might be important for the regulation of hESC proliferation and migration. In contrast to E2, G1-induced ERK activation was sufficient for the regulation of hESC migration. The real time Q-PCR (preliminary data) in GPR30-knockdown hESC showed that we could reduce the mRNA-level of GPR30 effectively in comparison to the controls. Surprisingly, GPR30-knockdown reduced the mRNA-levels of ERα and ERβ simultaneously in the absence of hormonal stimulation.

Conclusion

In conclusion, we provide evidence for a complex regulation of the non-genomic activation of ERK in normal hESCs. Furthermore we propose a working model, explaining how this is achieved in our *in vitro* cell system. E2 binds to ERα/ERß and/or GPR30 and these receptors then form an "activation unit" with c-Src family kinases and G-proteins. This results either directly or via transactivation of EGFR in the upregulation of the levels of pERK. It then goes into the nucleus and acts on its downstream target c-jun (Fig. 1). The future perspective is to evaluate the proposed working model in human endometrial stromal cells (hESC) after the GPR30-knockdown.

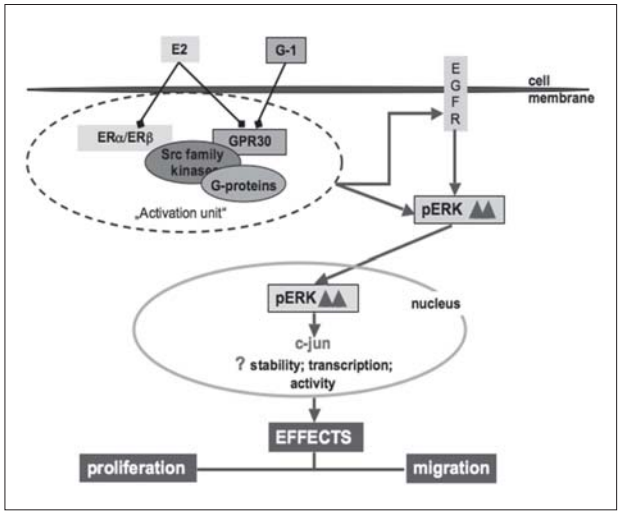

Fig. 1 - Proposed Working Model for the signal transduction upon E2- or G1-stimulation via ERα, ERβ and GPR30 in human endometrial stromal cells.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Two years monitoring of hormone endometrial regulation in perimenopausal dysfunctional uterine bleeding

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Background

Endometrial hormone regulation is an attempt for non-surgical, conservatory management of dysfunctional uterine bleeding (DUB).

Objectives

Endometrial regulation after two years of hormone treatment (HT) different by administration route in perimenopausal dysfunctional uterine bleeding (DUB).

Study design

Pathological endometrial prospective assessment during January 2008-December 2010, in two in-patient gynecology departments of 35 perimenopausal DUB, hormonally treated if accomplished inclusion/exclusion criteria (Table 1), compared to 15 untreated.

Endometrial samples [collected at enrollment, after one, and two years of HTin the 9-11th days of progesterone or in the 22-25th days of HT] are stained with commune histological techniques (hematoxyline- eosine, van Gieson). Endometrial assessment is done by a single, independent pathologist, using the WHO classification (1994) and the criteria of Endometrial Collaborative Group (2000) for endometrial hyperplasia (EH) and endometrial intraepithelial neoplasia (EIN) or endometroid neoplasia (European Study, 2001).

Endometrial changes are appreciated in the terms of hyperplasia prevention, on epithelium, glands, stroma, vessels.

TABLE 1 - INCLUSION/EXCLUSION CRITERIA.

Inclusion	Exclusion
• 40-50 years	• submucous leiomyomas
• minimum 3 months with DUB	• endometrial hyperplasia with atypia
• no previous HT for at least 3 months	• history of endometrial, breast cancer or other hormone dependent tumor
	• ovarian cysts
	• suspect mammographic changes one previous year
	• actual pelvic inflammatory disease
	• actual/history of thromboembolism
	• severe liver disease, jaundice or generalized pruritus during pregnancy
	• Dubin Johnson/Rotor Syndrome
	• Von Willebrandt disease
	• congenital disturbances of lipids metabolism
	• history of gestational herpes
	• pregnancy aggravated othosclerosis
	• intolerance to ethynil-estradiol/estradiol, progestogens/progesterone

Pathologic analysis of endometrial structures

- *Architecture* and *cytological analysis* of glandular epithelium is done looking for atrophic, weak proliferative, secretory endometrium, hyperplasia: mitosis for proliferative effect, subnuclear glycogen accumulation and intraluminal secretions for secretory changes. Mitosis *per se* are not quantified, the categories 2-3 (WHO, 1997) do not have mitosis by definition. The regulation effect of progesterone is analyzed for the degree of proliferation suppression- mild, moderate, strong, when is not possible an accurate assessment of secretory transformation treatment induced.
- *Stromal analysis:* characteristic aspects for perimenopause and menopause/postmenopause: the fibroblast-fibrocytic proliferation-marked (MP) and moderate (mP), the fibrosis, and the presence of inflammatory elements (I). In oder to differentiate and discriminate between EH and EIN- a premalignant lesion, it is specially assessed the stromal volume. EIN diagnosis is based on the presence of cytological demarcation, crowded gland architecture, minimum size of 1mm, careful exclusion of mimics, and diminution of stromal volume to less than approximately half of the total sample volume (a new architectural criterion for EIN) (Mutter GL, 2000, 2002; Dietel M, 2001). Most EIN lesions have been diagnosed as atypical endometrial hyperplasias in the WHO system.
- *Vasculature analysis:* vessels' number on examined fields: high (HV) and low number (LV), and subepithelial hemorrhages (H).
- EIN diagnosis is based on presence of cytological demarcation, crowded gland architecture, minimum size of 1mm, mimics exclusion, diminution of stromal volume to less than approximately half of the total sample volume (Mutter GL, 2000, 2002; Dietel M, 2001).

TABLE 2 - CLASSIFICATION OF BIOPSIES (WHO, 1997 AND ENDOMETRIAL COLLABORATIVE GROUP, 2000).

0= no tissue
1= insufficient tissue for diagnosis
2= atrophic/inactive endometrium
3= secretory endometrium
4= normal proliferative endometrium
5= endometrial hyperplasia (EH): simple, complex without and with atypia
6= endometrial intraepithelial neoplasia (EIN)
7= carcinoma (endometrioid, non- endometrioid type)

Statistic analysis

Paired-sample *t* test.

Results

The patients are registered in Table 3, with the demographic characteristics in Table 4.

TABLE 3 - PATIENTS AND TREATMENTS.

Group 1: 15 cases	transdermal (trd) 17-β-E2 gel (Oestrogel®) 1g/day, delivering 1mg E2, 28 days and vaginal micronised progesterone (VMP-Utrogestan®) 200mg/day for 12 days, from the 14th day of 17-β-E2
Group 2: 10 cases	200 mg/day VMP for 12 days from the 14th day of menstrual cycle
Group 3: 10 cases	Ethynil-estradiol (0.030mg) + Dienogest (2 mg) for 21 days (Jeanine®)
Controls: 15 cases	Untreated perimenopausal patients, who refused treatment

The demographic characteristics are similar, with the exception of parity (more multiparous treated) and smoking (more controls).
At enrollment microscopy registered similar endometrial aspects: hyperplasia (complex hyperplasia without atypia: 1 case in group 1 and controls; simple hyperplasia without atypia: 16-45.7% treated, and 5-33.3% controls), weak proliferative (16-45.7% treated, and 8-53.3% controls), secretory (1 in group 1, and controls), 1 case with endometrial polyps in the second group.

TABLE 4 - PATIENTS CHARACTERISTICS.

	Treated	Controls
Number	35	15
Age (yrs)		
Average	47.3	48
Variation	45-52	46-55
Weight (kg)		
Average	59.6	59.7
Variation	44-91	50-79
Height (cm)		
Average	160.8	160.6
Variation	135-168	154-164
Parity:		
Nuliparous	1	5
Multiparous	34	10
Smokers	2	5

After 12 months HT: 4-40% inactive/atrophic endometrium in O vs 3-12% in NO groups - p<0.01; secretory changes in NO groups (21-80.6%) vs O (5-64%) -p <0.01, with accentuation after 2 years (atrophy: O 9-47.3% vs NO 6-26% -p<0.01; secretory: NO 16-66.6% vs O 1-10.0%- p<0.01); special mention when VMP (8- 80%); 1 case with simple hyperplasia without atypia after 24 months on transdermal estrogen (proliferative endometrium at prestudy) vs 7-46.6% controls (among which 2 are complex hyperplasias without atypia, at prestudy being simple hyperplasias without atypia). In controls: from 8 proliferative endometrium at prestudy, 5 are registered after 2 years. No carcinoma registration.

The analysis of the three hormone regimens from the baseline is focusing on secretory and atrophic changes, appreciated as safe for endometrium health. After 12 and 24 months treatment there are recorded:

- low grade secretory changes, characterized by cytoplasmic vacuolization: 74.3%, respective 48.7%;
- atrophy/inactive endometrium: 20.0%, respective 40.0%;
- inadequate progestogenic response: 6 cases;
- proliferative endometrium: 3 cases at 12, 1 case at 24 months;
- simple hyperplasia without atypia:1 case at 24 months;
- increase of blood vessels number, and sub-epithelial hemorrhages (more frequent at 24 months).

The NO groups have at 12, and 24 months marked fibroblastic-fibrocytic proliferations, fibrosis reduction, and increase of granulocytes inflammatory cells - specially in the first year; the group on COC has marked stromal edema after the first year (Table 6).

Proliferative endometrium is more frequent after NO vs O: 8.0% vs 9% at 12 months (4.0% vs 0 at 24

TABLE 5 - ENDOMETRIAL BIOPSIES.

	Group 1 E_2 trd + VMP			Group 2 VMP			Group 3 E_2 + Dienogest		
	months			months			months		
	0	12	24	0	12	24	0	12	24
Insufficient tissue	0	0	0	0	0	0	0	0	0
Atrophy/inactive	0	2	3	0	1	2	0	4	9
Proliferative	8	1	1	6	1	0	2	1	0
Secretory	1	13	8	0	8	8	0	5	1
Hyperplasia	6	0	1	4	0	0	7	2	0
Others	0	0	0	0	0	0	1	0	0
Carcinoma	0	0	0	0	0	0	0	0	0
Total	**15**	**15**	**15**	**10**	**10**	**10**	**10**	**10**	**10**

	Control			Total		
	months			months		
	0	12	24	0	12	24
Insufficient tissue	0	–	0	0	0	0
Atrophy/inactive	0	–	2	0	7	15
Proliferative	8	–	5	24	3	3
Secretory	1	–	1	2	26	25
Hyperplasia	6	–	7	23	0	8
Others	0	–	0	1	2	0
Carcinoma	0	–	0	0	0	0
Total	**15**	**–**	**15**	**50**	**35**	**50**

TABLE 6 - STROMAL AND VASCULATURE CHANGES.

	Stroma				Vasculature		
Number of Cases/Percentage	**MP**	**mP**	**F**	**I**	**HV**	**LV**	**H**
Enrolment = 35	19/52.3	21/40.8	4/7.6	33/60	23/47.6	19/52.3	7/13.8
12 months = 35	31/88	3/12	1/2	21/70	24/82	7/18	8/24
24 months = 35	30/87	3/12.5	0	21/70	30/82.5	6/17	9/25

Legend: MP=marked, mP=moderate fibroblast-fibrocytic proliferation; F=fibrosis; I=inflammatory elements; HV=high; LV=low number of vessels; H=sub-epithelial hemorrhages.

months); proliferative aspects are more frequent at 12 than at 24 months.
Secretory changes are more frequent after NO: (80.6%) vs O (64.0.%) at 12 months, and 66.6% vs 10.0% at 24 months, p<0.01.
Atrophy is predominant after oral HT vs NO: p<0.01 (40.0% vs 12.0% at 12 months, 90.0% vs 20.0% at 24 months).

Discussion

Dysfunctional uterine bleeding is defined as abnormal uterine bleeding caused by a hormonal mechanism. Any alteration of the normal menstrual cycle mechanisms can lead to steady-state estrogen production and DUB.
Perimenopause is the period of 2-8 years preceding menopause and one year after the final menses (WHO), and a better practical definition is the phase preceding the onset of menopause, generally occurring around 40-50 years of age (beginning at age 47.5, lasting for 4 years) during which the regular cycle of a woman transitions to a pattern of irregular cycles, because of luteal phase insufficiency, or anovulation.
The ages of patients enrolled in this study were in years of menopausal transitions, the histopathological reports of endometrial pattern were in the limits of abnormal dysfunctional bleeding: weak proliferative (16- 45.7% in treated, and 8- 53.3% in controls), hyperplasia without atypia (simple: 16- 45.7% in treated, and 5- 33.3% in controls; complex: one case in treated and one case in controls), and. seretory endometrium (one case of in group 1 and in control), one case with endometrial polyps in the second group.
Our analysis regarding endometrial stroma has revealed two important aspects to be discussed: 1) the increase of the fibroblast-fibrocytic proliferation in the stroma and the reduction of fibrosis in all treated cases, indirectly the maintain of the stromal volume (an important criteria when discussing about endometrial intraepithelial neoplasia).
2) the increase of the granulocyte inflammatory reaction is like a pseudodecidualisation and not a sign for endometritis, which need to discover the presence of periglandular plasma cells and leukocytes. In the natural menstrual cycle the presence in the stroma of these leukocytes infiltration is normally in the premenstrual phase, when starts the estrogen and progesterone decline.
In UK a recent endometrial analysis for perimenopausal dysfunctional bleeding (84 cases) (Michail G, Karahaliou A, Skiadopoulos S, et al., 2007), besides proliferative endometrium (9.79%), simple hyperplasia (8.53%), there were found endometrial inflammation (20.7%), endometrial atrophy (21.9%).
The risks revealed by HERS, WHI for oral HT imposed the analysis of endometrial effects of other regimens with: only low dose estrogens, low dose synthetic estrogens as in combined oral contraceptives, new types of progestogens (gonan, norpregnan, spironolactone derivates- in reduced doses parallel with reduced doses of estrogen) as alternatives to medroxyprogesterone acetate, norethisterone or non- oral route of administration [transdermal estrogens, (total) transdermal estrogens and progestogens, or vaginal micronized progesterone] for prevention of endometrial hyperplasia, and carcinoma.
Oral contraceptives exert a predominant progestational effect on the endometrium, inducing an arrest of glandular proliferation, pseudosecretion, and stromal edema and granulocytes, progressive endometrial atrophy after prolonged use, and indirect reduction of endometrial hyperplasia/carcinoma.
We consider that oral combined contraceptive methods, progesterone/other HT, recommended during menopausal transition, to regulate the menstrual cycle, and to protect from excessive endometrial proliferation/ hyperplasia/ cancer can be continued in menopause, in order to use the "window of opportunity", to control climacteric complains. These are the reasons for this study of vaginal micronized progesterone, or transdermal estrogen plus vaginal micronized progesterone, versus the oral combined contraceptive regimen with ethynil estradiol plus dienogest for DUB in perimenopause.
The endometrial protection from proliferation, hyperplasia, or carcinoma was ensured by continuous administration of ethinyl estradiol and dienogest, and by the 12 days of 200 mg micronized progesterone vaginally administrated alone, or sequential to transdermal estradiol; in all the cases, and specially in those with hyperplasias without atypia at enrolment, we used the WHO's classification (1994) and the The Endometrial Collaborative Group (Mutter GL, 2000), regarding EIN.
The case with simple hyperplasia without atypia after transdermal estrogen and sequential vaginal micronized progesterone for 12 days (incidence 1/35 cases) is inside the oncological safety (2 cases/100 women year), if we appreciate the considerations of de Lignieres B (1999) regarding the risks with micronized progesterone, or the latest review regarding endometrial hyperplasia (Palmer EJ, Perunovic B, Tidy AJ, 2011). EIN lesions originate focally and expand in size over time, in keeping with a proliferative monoclonal origin. EIN arises through complex interactions involving the sequential accumulation of genetic damage

in endometrial glands and the positive selective pressure of unopposed estrogen.
EIN is characterized by closely packed glands (volume percentage stroma <55%) with cytology that is clearly demarcated from that of the adjacent field. A minimum homogeneous field of cytologically demarcated glands is required to accurately assess the architecture diagnostic of EIN, and morphometry-diagnosed lesions with a largest diameter of at least 1 to 2 mm have previously been shown to predict the relevant clinical outcome of concurrent or future endometrial adenocarcinoma.
Regarding the pathological characteristics of EIN, the case with hyperplasia is conserving the stromal volume due to marked broblastic- fibrocytic proliferation.
At authors knowledge it is no study assessing endometrial safety on the association of transdermal estrogen and vaginal micronized progesterone in perimenopausal women. There were assessements of endometrial safety in postmenopause of the transdermal associations, such as:

1. Estradiol 50 µg with norethindrone acetate 140, 250, or 400 µg /day (CombiPattch Study Group, Archer DF, Furst K, et al, 1999);
2. Estradiol 0.045 mg/day with levonorgestrel 0.015, 0.030, and 0.040 mg/day (Shulman LP, Yankov V, Uhl K, 2002);
3. 17β estradiol: 4.4/2.74 and levonorgestrel: 4.5/3.75mg (Dando TM, Perry CM, 2004);
4. Estradiol 50 µg/day and NETA, 170 µg/day or 350 µg/day, either continuously or sequentially (Ylikorkala O, Rozenberg S, 2000);
5. Estradiol 25 µg/day and NETA 125 µg/day (Estalis 25/125 Study Group: Samsioe G, Dvorak V, Genazzani AR, et al, 2007).

All these researches concluded the endometrial safety: no hyperplasia.

Conclusions

- NO and oral HT induce similar endometrial aspects, but not identic to those of the reproductive age.
- Non-oral hormone therapy is endometrial safe for 2 years.
- Proliferative endometrium is more frequent after NO vs O: P<0.01; secretory changes are more frequent after NO: P<0.01, and atrophy is predominant after oral HT vs NO: P<0.01.
- The highest percentages of secretory changes are after VMP.
- The case with simple hyperplasia without atypia after transdermal estrogen plus sequential vaginal micronized progesterone (incidence 1/35 cases) is inside the oncological safety (2 cases/100 women year).

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

In vitro and in vivo effects of inositol in patients with PCOS

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine-metabolic diseases, affecting 6-10% of women in fertile age (1). Increasing evidence supports the central role of insulin resistance (IR) and compensatory hyperinsulinemia in the pathogenesis of PCOS and in the increased cardiovascular risk (2). Previous studies have found a deficiency and/or altered metabolism of D-chyro insoitol (DCI), a specific putative insulin mediator, and have shown that oral administration of DCI to PCOS women improves IR, ovulatory function and hyperandrogenism (3-6).

PCOS is associated with a proinflammatory state and an elevated production of reactive oxygen species (ROS) is involved in the pathogenesis and in the future complications of the syndrome (7). We recently demonstrated that patients affected by favism or endometriosis show high oxidative stress in the erythrocyte membrane, characterized by increased tyrosine phosphorylation (Tyr-P) level of membrane band 3, which represents a useful parameter in the evaluation of oxidation-related cell damage (8,9).

The aim of this study was to investigate oxidative-related alterations in erythrocyte membrane status and in vitro and in vivo anti-inflammatory effects of inositol in PCOS women.

For in vivo study, we enrolled 26 PCOS patients, 22 to 30 years of age, who were randomized to receive MYO dietary supplement powder 1200 mg/day (Redestop, Progine, Firenze) (n=18) or matched placebo powder (n=8) for 12 weeks.

All patients met the inclusion/exclusion criterion of the ESHRE/ASRM consensus for diagnosis of PCOS (11). Before (T0) and after (T1) MYO treatment, blood samples were collected in fasting conditions to measure serum testosterone, androstenedione, and glucose and insulin during oral glucose tolerance test (OGTT). Values are expressed as the area under the curve (AUC) of glucose and insulin, respectively. A peak of serum insulin value during OGTT > 70 mU/L was considered an IR index. Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR) was also used.

For the evaluation of the oxidative-related alterations we analysed Tyr-P levels induced by 1.5 mM diamide incubation for 30 min in purified human erythrocytes from PCOS patients (in vivo study), or from both patients (n=6) and healthy volunteers matched for age and BMI (n=6) after a pre-treatment in the absence or presence of 2 mM inositol (in vitro study).

Membranes were recovered, analyzed by Western blotting and immunorevealed with anti P-Tyr antibodies.

The Tyr-P value of diamide-treated erythrocytes before (T0) and after (T1) in vitro or in vivo treatment was calculated as the ratio to the Tyr-P level of diamide-treated erythrocytes obtained in healthy controls (chosen as arbitrary comparison unit). To express a standardized measure of Tyr-P variation after in vitro or in vivo treatment [Tyr-P variation index, **Vi(Tyr-P)**] with respect to baseline value, the following formula was used:

$$Vi(Tyr\text{-}P) = 1 - (Tyr\text{-}PT1/Tyr\text{-}PT0)$$

Comparisons were obtained with Student's *t*-test for paired or unpaired data, as appropriate. Data are expressed as mean±SD. $p<0.05$ (two-tailed) was considered significant.

Baseline clinical characteristics and haematochemical parameters determined in the two groups of PCOS showed no significant differences. Following the 12-week treatment, comparing the two groups, in the MYO group we observed a significant reduction of

BMI (p<0,02), serum values of testosterone (p<0,0001) and androstenedione (p<0,003), fasting insulin (p<0,003), AUC insulin after OGTT (p<0,006) and HOMA-IR (p<0,001). No significant changes were observed in the placebo group.

Increased systemic oxidative stress was studied on erythrocytes membrane status by Tyr-P levels of band 3. In baseline conditions, in absence of diamide stimulation Tyr-P could not be detected in erythrocytes from either patients or controls, but when PCOS RBC were incubated with diamide, membranes showed a significantly higher Tyr-P levels (185±20% compared to healthy controls, considered as 100%).

Both in vitro (p<0,02) and in vivo (p<0,05) MYO treatment significantly reduced Tyr-P levels of band 3 (17±2% and 35±7% respectively). No variations were found in the placebo group.

To assess interrelationships between RBC parameters and hormonal/metabolic data at T0 and T1, a linear regression was performed and the strength of correlation was assessed by Pearson's correlation coefficient r (by ANOVA). In the MYO-group we obtained a significant correlation between serum testosterone and Tyr-P levels (p<0.05). Comparison of the two regression lines, at T0 and T1 respectively, revealed a significant difference between the intercepts in MYO-treated patients (p<0.0001); the slope remained the same, indicating that, although phosphorylation kinetic mechanisms were unaffected by MYO administration, they were quantitatively reduced. The same analysis, applied to the placebo group, confirmed the significant correlation between Tyr-P and serum testosterone levels only at T0 (p=0.0032). A significant correlation was detected also between insulin AUC and Tyr-P, but only at baseline (p<0,05). After MYO treatment, the concomitant reduction found for Tyr-P and insulin AUC values produced a clustering of data that abolished the regression of the two variables.

Our study underlines that PCOS patients suffer from systemic redox impairment, as indicated by oxidative-related alterations in erythrocytes band 3 Tyr-P of all patients in basal conditions. Interestingly, inositol treatment both in vitro and in vivo positively affected the oxidative status of RBC, as shown by the significant reduction in band 3 Tyr-P levels. The present study also confirms the beneficial effects of MYO treatment on ameliorating hyperandrogenism and IR. In particular, the significant reduction of Tyr-P levels in RBC and insulin AUC found at T1, confirms the strength relationship between IR and oxidative stress in the pathogenesis of PCOS and that MYO uptake can modulate insulin intracellular pathways and oxidative stress induced by the inappropriate hyperinsulinemic response linked to IR.

Another novel data is the significant correlation between Tyr-P and testosterone levels at T0 and, more interestingly, at T1, suggesting that alteration of the phosphorylation process of erythrocyte band 3 is mediated by higher testosterone levels in serum. We hypothesize that administration of MYO improves band 3 Tyr-P through a decrease of testosterone level (11).

In conclusion, Tyr-P process could be very useful in monitoring patients' conditions and in choosing the adequate therapy. MYO can be considered an alternative to metformin for PCOS.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Functional role of peroxisome proliferator-activated receptors gamma in glucidic metabolism of granulosa cells from normal and polycystic ovary syndrome women

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Introduction

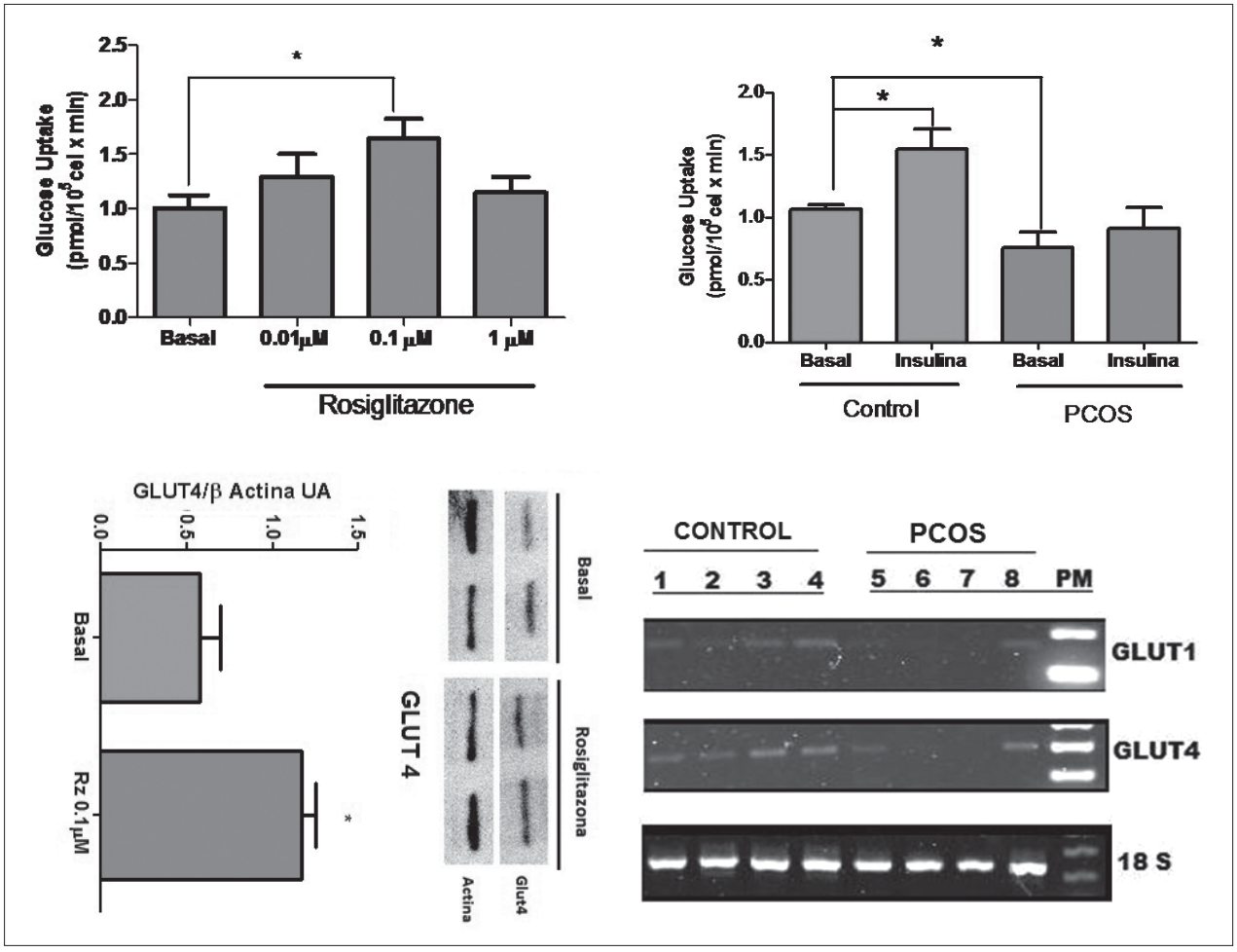
Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders that affect 5-10% of women during reproductive age. Being the most frequent cause of anovulatory infertility and hyperandrogenism. In addition 40 to 50% of these patients exibit hyperinsulinemia and insulin resistance.
Adipocytes and muscle cells of PCOS women have a reduced uptake of glucose and expression of glucotransporters, without changes in receptor number or affinity for insulin. It is thought that insulin resistance of these tissues is associated with impaired post-receptor signaling. PPAR γ are key transcription factors that regulate metabolism of glucose and Insulin. Rosiglitazone belongs to the family of thiazolidinediones, it is a potent selective agonist of PPAR γ receptors, regulating the expression of genes involved in lipid and glucose metabolism. Glitazones increase the incorporation of glucose-insulin-dependent in cultured myocytes and adipocytes modulating the expression of GLUTs.
Conversely, the glucose metabolism and the insulin signaling pathway in granulosa cells (GCs) of PCOS patients are virtually unknown. The aim of this study was to determine the role of PPAR γ and its agonist Rosiglitazone in the metabolic pathway of Insulin in granulosa cells of PCOS patients.

Methodology

Follicular fluid (FF) from the first aspirated follicle and granulosa cells (GCs) from the whole follicle aspirated were collected from PCOS (n= 15) patient participating in the IVF program of our institute (Institute of Maternal and Child Research IDIMI, Faculty of Medicine University of Chile. Campus Centro). Similar samples were collected from normal women involved in our IVF program due to male factor infertility and were used as control group (n=18).
GLUTs and PPAR γ genes expression were evaluated by qRT-PCR, the proteins were localize by immunocistochemistry, (ICQ) inmunofluorescence and protein levels were determined by Western blot. Glucose was measure in follicular fluid by glucose oxidase technique and its uptake was assessed by incorporation of 2-desoxy-[^{3}H]-glucose (2-DG) in both basal and stimulated condition (Insulin 100 ng/ml), as well as in pre incubated cells with Rosiglitazone (0,01-1 μM). 2-DG can be uptake by the cell but can't be metabolized or degraded so we can measure the exact amount of glucose that the cell incorporates.
Data are presented as mean ± SEM. Comparison between groups was performed using analysis of variance (ANOVA) in conjunction with Turkey test or Student's t-test. The result was considered statistically significant when p values ≤0.05.

Results

Both groups were similar in age and BMI. PCOS group were hyperandrogenic and had insulin resistance with compensatory hyperinsulinemia.
Glucose levels in follicular fluid of PCOS follicles were significant higher compared to the control group (41,14±5,1 v/s 18,33±2,8 mg/dl p<0,05). PCOS GCs show a marked decrease in the expression of mRNA of GLUT1 and GLUT4 compared to the control group (p<0,01). The same decrease was observed at the pro-

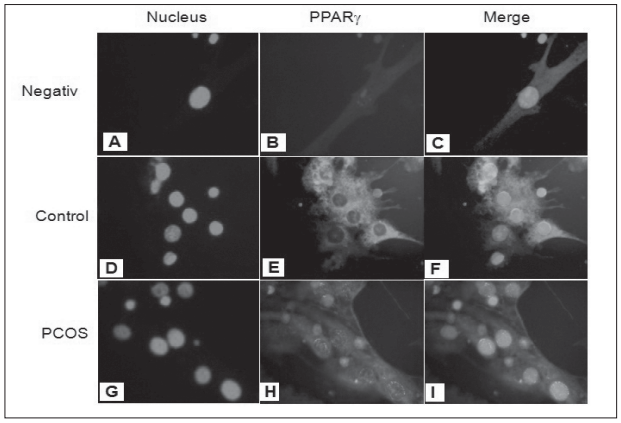


tein expression level in the PCOS CGs determined by ICQ using Expression Level Score (ELS).

Basal glucose uptake diminished signifincantly in the PCOS GCs compare with control GCs (0,66±0,1 v/s 1,016±0,017 pmol/10^5 cel/min $p < 0.05$). The addition of insulin (100 ng / ml) to the culture medium for 1 hour enhanced the glucose uptake in the control group without modification in the sugar uptake of PCOS GCs.

PCOS GCs expressed significant lower abundance of PPAR γ mRNA ($p < 0,05$) and the PPAR γ protein is mainly localized at the nuclear level ($p < 0.001$) compare with control. The incubation with Rosiglitazone 0.1 µM for 12 hours significantly improved the incorporation of 2-desoxy-[^{3}H]-glucose ($p < 0,05$) in PCOS GCs and increased the levels of GLUT4 mRNA and its protein.

The results of this study show that PCOS GCs have different metabolic and molecular profile than CGs of normal women. Glucotransporters are decreased in PCOS GCs and revealed resistance to insulin modulation.



In turn, the followig in vitro data indicate that the activation of PPAR γ by the pharmacological agonist Rosiglitazone improves glucose uptake in the granulosa cells of PCOS women. This effect is probably driven by the increase of GLUT4.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Different prolactin isoforms in gynaecological pathologies

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Introduction

Human Prolactin (PRL) is a proteic hormone produced by the anterior pituitary gland. The secretion is regulated by different endogenous factors: dopamine is the predominant prolactin-inhibiting neurotransmitter, serotonin and TRH stimulate secretion of PRL (1).
PRL circulates in three different isoforms: monomeric (mPRL) with a molecular weight of 23kDa, that has the highest receptor affinity and biological activity; Big-PRL, which has a molecular mass of 50kDa and is a dimer of PRL; macroprolactin (MPRL) with a molecular weight greater than 150kDa and an abstent bioactivity. MPRL is a complex of mPRL and antibodies (IgG, IgA, IgE) and different glicosilation (2).
Moreover, post-transcriptional modification of pituitary PRL generates a variety of additional species, including glycosylated and phosphorilated variants, together with 16kDa proteolysed form (3).
PRL promotes breast development and stimulates milk production, but PRL hypersecretion interferes with pulsatile gonadotropin releasing hormone secretion, resulting in mestrual irregularities and gonadal dysfunction. Macroprolactinaemia is found in 9.6-29% of patient with hyperprolactinaemia (4).
The aim of this study in to evaluate the secretion of the different isoforms of PRL and their clinical impact on gynaecological pathologies. In particular, mPRL and MPRL's behavior were studied in order to make a differential diagnosis based on clinical signs and values of different isoforms.

Materials and methods

Eighty-two patients with hyperprolactinaemia and irregular menses were admitted to the Department of Endocrinology of University of Brescia/Spedali Civili between 2008 and 2010. All patients underwent to endocrine evaluation: hormone evaluation, MPRL assay (precipitation with Poliethylen Glycole) and Magnetic Resonance Imaging of the turcic sella.

Results

Diagnosis of prolactin secreting adenomas was confirmed in 49% of patients, whereas in 51% there was a dysfunctional hyperprolactinaemia. In this second group the diagnosis was Polycystic Ovary Syndrome (PCOS) in 48% of patients, iatrogenic anovulation in 36%, obesity/metabolic syndrome in 11% and endometriosis in 5%.
Secondary amenorrhea was present in 97.5% of patient with adenomas, whereas in 38.1% of dysfunctional patients. Oligoamenorrhea was present in 47.6% of dysfunctional forms.
Galactorrhea was found in 87.5% of women with prolactinomas and in 9.5% of dysfunctional patients (Tab. 1).
The highest level of total PRL were found in patients with adenomas, showing increased prevalence of mPRL than MPRL. In dysfunctional pathologies MPRL was the prevalent isoform (Tab. 2).

Conclusions

Different isoforms of PRL are associated to different gynaecological pathologies. Prolactinomas are associated with a MPRL/PRLm ratio <1, galactorrhea and amenorrhea, whereas dysfunctional pathologies are associated to an elevation of MPRL and MPRL/PRLm ratio >1. These forms of hypermacropralctinaemias do

TABLE 1.

	Group 1 (Disf. HyperRPL)	Group 2 (Adenomas)
Number	42	40
Age (years)	28.9±11.4	30.0±10.2
BMI (kg/m^2)	25,6±6,1	24,5±3,1
Regular menses	2 (4,7%)	0
Irregular menses	40 (95,3%)	40 (100%)
Oligoamenorrhea	20 (47,6%)	1 (2,5%)
Amenorrhea	16 (38,1%)	39 (97,5%)
Hyperpolymenorrhea	4 (9,5%)	0
Galactorrhea	4 (9,5%)	35 (87,5%)

TABLE 2.

	Group 1 (Disf. HyperRPL)	Group 2 (Adenomas)
Total PRL (ng/ml)	68.2±10.5*	80.1±20.5
MPRL(ng/ml)	45.9±15.8*	20.2±8.8
mPRL(ng/ml)	22.4±11.2*	65.3±30.2

Student t test P<0.05 group 1 vs group 2.

not have clinical consequence on the ovary. In these patients the menstrual irregularities were caused by other endocrinological pathologies.

In conclusion, differential diagnosis of hyperprolactinaemia could be made with clinical signs and values of different isoforms of PRL.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Long term morbidities of polycystic ovary syndrome (PCOS)

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive-age women, affecting 5 to 10% of them. Although clinical features of the syndrome are heterogeneous and extremely different between individuals, Rotterdam Consensus in 2003 established that diagnosis should be based in presence of at least two of the three major criteria, including: chronic oligo-anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovarian morphology based on ultrasound scan, excluding other androgen excess causes.
Aetiology of the syndrome is still controversial, but insulin-resistance and hyperandrogenism play a central role in the development of the syndrome, determining clinical signs, infertility, obesity, hyperglycaemia and dyslipidaemia, which are potential risk factors for these patients, and are involved in long term consequences, such as cardiovascular events and cancer.
Hyperandrogenism is the key feature of PCOS, and it is primarily due to an excessive androgen production in ovaries, mainly caused by an increased LH stimulation (abnormal LH pulse secretion) and by hyperinsulinemia. Insulin resistance improves the action of LH and increases with the obesity. So, insulin and LH in synergy contribute to stimulate androgens production.
The aim of this study is to evaluate long term morbidities of PCOS. In particular, the relationship between the syndrome and the development of cardiovascular, metabolic, neoplastic and pregnancy events was studied.

Materials and methods

Among patients admitted from 80' to the Department of Gynaecological Endocrinology of University of Brescia/Spedali Civili, 515 PCOS underwent long term follow-up of 25.3±4.8 years (17-37 years).

TABLE 1 - METABOLIC AND CLINICAL FEATURES OF PCOS AT ADMISSION.

	PCOS (n = 515)	%
Mean age (yr)	22.3±4.8 (13-36)	
Mean BMI (kg/m²)	23.12±4.6	
Underweight	47	9.1
Normal weight	330	64.1
Overweight	89	17.3
Obese	49	9.5
Menarche (yr)	12.8 ±2.7 (9-17)	
Cycles		
Oligomenorrhea	306	59.6
Amenorrhea	139	26.9
Regular	45	8.7
Polimenorrhea/Menometrorrhagia	25	4.8
Hypercholesterolemia	76	14.7
Hypertrigliceridemia	29	5.6
Glucidic intollerance	9	1,7
Blood pressure (mmHg)		
Systolic blood pressure	118.5±12.9	
Diastolic blood pressure	72.9±9.95	
Patients with acne	186	36.1
Patients with hirsutism	262	50.9
Parity (women with children)	18	3.4
Miscarriage	21	4.1

Results

PCOS women show a progressive weight gain, with a greater incidence of obesity from 26.8% to 41.5% during life, due to abnormalities in insulin action and secretion. In fact insulin resistance is associated with the development of abdominal obesity.
Oligomenorrhea is the most common feature in patients with PCOS, but during years spontaneous regularization of menstrual cycles was obtained in 64.4%, especially after pregnancy.
This is probably due to adrenopause process (a gradual reduction in adrenals androgens production, which began by the third decade of life) and to pregnancy, which represents an essential moment in the restoring of hormonal balance, due to the estrogenic milieau that contrasts androgenic effects.
Chronic anovulation is one of the most important cause of infertility. In fact, it was present in 40.7% of patients, and 65% of these required ovulation induction (using clomiphene citrate). 21.4% of PCOS was clomiphene resistant and were not able to get pregnancy.
PCOS has an increased risk of pregnancy adverse events (higher rate of miscarriage, gestational hypertension, preeclampsia and premature delivery (1)). This may be due to hyperinsulinaemia and hyperandrogenemia that can adversely affect endometrial and ovarian function. In addition, endothelin-1 and PAI-1 (plasminogen activator inhibitor-1) which are present in high levels in PCOS, are associated with placental thrombosis and fetal loss (2).
Insulin-resistance and hyperandrogenism modulate plasmatic levels of some haemostatic factors and they improve lypolisis, increasing the release of free fatty acids, secretion of VLDL and triglycerides (3). For these reasons, cardiovascular risk factors are increased in PCOS versus general population (4,5). In particular, diabetes was present in 5.9% of patients, hypertension in 20.4%, hypercholesterolemia in 27.3% and hypertrigliceridaemia in 4.2%. Despite that, no differences in major cardiovascular events (stroke, thrombosis, myocardial infarction) were found in treated PCOS when comparing with the general population, but the incidence of phlebitis was two times greater (6.9%), perhaps because of a long estroprogestinic use (6).
We did not found any dissimilarity in thyroid function between PCOS and general population (7).
No significant differences in benign gynaecologic pathology (uterin polyps, leiomyoms, ovarian cysts and breast fibroadenomas) seek out in PCOS. A condition of relative hyperestrogenism may increased these disorders, but estroprogestin therapies seems to have a protective effect.
Incidence of neoplastic pathologies (endometrial, breast, ovarian and colon cancer) was not different from local general population (6). This can be partially explaned by a prolonged estroprogestin treatment, which explicate a protective effect in some of those diseases. In fact, in patients who didn't submitted estroprogestin treatment the incidence was higher.

Conclusions

In conclusion, patients with PCOS showed infertility, menstrual irregularity, a greater rate of adverse pregnancy outcomes, predisposition to obesity and higher cardiovascular risk factors than general population, but cardiovascular events are not increased in treated patients. Furthermore cancer incidence is not significantly increased in treated PCOS than general population. Treatment of PCOS is also able to change or minimize the development of long term sequelae. Therapies prematurely introduced restored a proper hormonal balance before vascular and metabolic consequences occurred. Treating "the whole state" rather than "the disease" should be the principal on dealing with PCOS.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Premature ovarian failure and infertility: case report

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Introduction

Premature ovarian failure (POF) affects 1% of women and is defined as the occurrence of amenorrhoea lasting more than 6 months along with high levels of gonadotropins (follicle-stimulating hormone, FSH >40 mU/ml, in at least two occasions) which is observed before the age of 40 (1). The aethiology of POF is unclear in the majority of cases; nevertheless either genetic or acquired pathogenetic mechanisms should be theoretically implicated: genetic mechanism include mutations in genes coding proteins that are essential for ovarian development, whereas acquired mechanisms include the effect of various environmental factors that can cause POF through autoimmune (2-4) or viral (5-7) oophoritis, the effect of environmental toxins and iatrogenic factors (surgery, radiotherapy and chemotherapy). 42.9% of POF cases are characterized as idiopathic/sporadic, 7.8% of cases are caused by autoimmune multiple endocrinopathy, whereas 4% of the cases are iathrogenic. As expected, cases with extremely early POF experience the consequences of oestrogen deprivation with more detrimental impact.
POF is associated with higher risk of osteoporosis, changes in lipid metabolism, cardiovascular system diseases. Infertility is a severe problem, especially, if woman is younger than 30 years. There are different treatment possibilities; most often used is HRT together with glucocorticosteroids.
To solve infertility, donor egg is used together with man's semen. Usually POF is in 1-5% women aged 20-35, when the donor's egg psychologically is difficult to accept.

Methods

We report two clinical cases (patient A and B). Both patients were younger than 37, with proven POF – 6-8 months amenorrhoea, FSH>70 U/L, estradiol ≈ 30 pg/ml, ovarian US (some little follicles 2-4 mm in diameter), infertility. Both patients agree that their data will be used on this publication.

Results

Pacient A
Anamnesis
Pacient A, age 28, born as third sister in family. Both sisters have irregular menstrual cycle, often only with oval contraceptives (OC), have no children, are married. One sister is proved to have POF.
Menarche since age 16, periodical irregular menstruations, amenorrhoea since the age of 26.
Infertility five years. For cycle regulation used OC or Duphaston. No hormonal investigations were done at that time.

Complaints (on admission in 2008)
Pacient had anenorrhoea for 2 years, hot flushes, sweating, palpitations, vaginal dryness and infertility for five years.
Investigations: Per vaginam: atrophic colpitis, small uterus. US – linear endometrium, some pointlike follicules in ovaries.
Hormonal analyses:
FSH – 55,9 U/L, LH – 1,3 U/L, E2 < 20 pg/ml, TSH – 0,838 mU/ml, thyroid antibodies – norm, PRL –

310 mU/l, ovarian antibodies - negative, AMH <0,16 ng/ml.
Husband spermogramme – normal

Treatement
Oral contraception with regular FSH controle. When FSH reached ~ 20 U/L on cycle days 3-5, then estrogen (4 mg Estradiol and Duphaston 10 mg) cyclic therapy was started.
Follicule formation was observed with US, as well as FSH and E2 levels detected. When the dominant follicule was forming in the middle of the cycle, reaching 18-20 mm, endometrium 8-10 mm, FSH – 20 U/L, the pacient received intramuscular Pregnyl 10000 units, continuing estrogen and progesterone therapy. Pregnancy occured only during the 4th cycle. During every cycle ovulation possibility was documented by hormonal analyses.

Treatement during pregnancy
After menstruation delay we checked HCG, estradion and progesterone.
Substitutional treatement during pregnancy was started at HCG 40-60 mU/ml. Pacient received Estrofem 2-4 mg and Utrogestan 400-600 mg/d. Hormonal level was checked regulary, fetal US was performed. Reaching pregnancy 10-12 weeks hormonal substitution was slowly decreased and after 16th week stopped. Genetical screening tests were normal.
12.07.2010 after 39 weeks of pregnancy a girl was born- weight- 3510g, Apgar scale 8/9 points. Probably pacient A had a geneticaly determined POF.

Pacient B
Anamnesis
In March 2010 pacient B, age 36 came in with complaints about having no menstruations for one year and infertility for three years.
Menarche since age 13, regular, 4/28 days.
In 2005 normal delivery, in 2006 missed abortion 7/8, followed by one year of infertility, after that unsuccessful IVF, then irregular cycle, followed by amenorrhoea. As a treatement she has been given cyclic HRT without effect and the infertility was continuing.

Investigations
FSH – 62,4 U/L, LH – 38,4 U/L, E2 <20 pg/ml, TSH – 1,5 mU/L, homocystein – 8,59 mmol/l, ATPO – 47 U/mL, ovarian antibodies- positive. USG – small uterus, some small foloculies in both ovaries.
Husband spermogramm – normal

Treatment
Oral contraception and dexmetasone 0.5 mg every day (to block ovarian antibodies), then with Estradiol growing endometrium till 10 mm and folicules till 20 mm, Pregnyl i/m 10000 units. Pregnancy occured during second cycle.

Treatment during pregnancy
Estradiol and Progesterone, at the begining small dose Dexametasone (0.25 mg per day till 8 gestation week to block ovarial antibodies), from week 14 no addicional therapy, because analyses and fetal growth was normal. Genetic tests normal, pacient refused amniocentesis.

Pregnancy result
14.08.2011, at 39th gestation week with spontaneus delivery boy 3500g, Apgare scale 9/10 was born. Now breastfeeding, child development normal.
For pacient B POF appeared as autoimune reaction to missed abortion after IVF.

Conclusion

It is desirable to try to obtain POF patients own ovulation with medication (HRT+glucoccorticosteroids), and only after failure send to IVF with donor eggs.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Parasitic myoma mimicking an adnexal mass

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Introduction

Uterine leiomyomas are the most common gynecological pelvic tumor, diagnosed in more than 25% of woman during their reproductive years and identified in about 80% of all histerectomy specimens (1). Retroperitoneal myoma are a rarer entity that constitute a diagnostic challenge for any clinician.
Although this entity was first described by Kelly and Cullen in the beginning of the twentieth century, few cases have since been reported, usually referring to individual case reports. Soliman et al. recently reviewed published literature and in December 2011 there were 51 cases reported, including their case report (2).

Case report

We report the case of a 54-year-old, multiparous, Caucasian female, who was referred to our out-patient clinic due to an adnexal mass with no pertinent clinical complaints. Her past medical history was relevant only for a vaginal hysterectomy due to fibroids, which she had undergone nine years previously.
On routine vaginal ultrasound a heterogeneous oval mass, with 52x39mm, was identified at the left ovary. Laboratory values were normal, including a CA 125 level of 7,5 U/ml.
An exploratory laparoscopy was performed and intraoperatively the ovaries where found to be of normal size without any macroscopic alterations. On further inspection of the pelvic cavity a well circumcised retroperitoneal mass was identified in the left pelvic side wall, behind the left ovary. Bilateral oophorectomy was then performed and the pelvic side wall tumor was totally excised with blunt dissection. The specimen was then removed through the umbilical incision in a LapSac® (Fig. 1).
The postoperative period was uneventful and patient was discharged on the first postoperative day.
The histopathological examination of the surgical specimen was consistent with a parasitic fibroleiomyoma, weighing 32g.

Discussion

Uterine myoma are a relatively common finding, but some rare extra-uterine growth presentations have been described in the literature, such as benign metastasizing leiomyoma, disseminated peritoneal leiomyomatosis, intravenous leiomyomatosis, parasitic

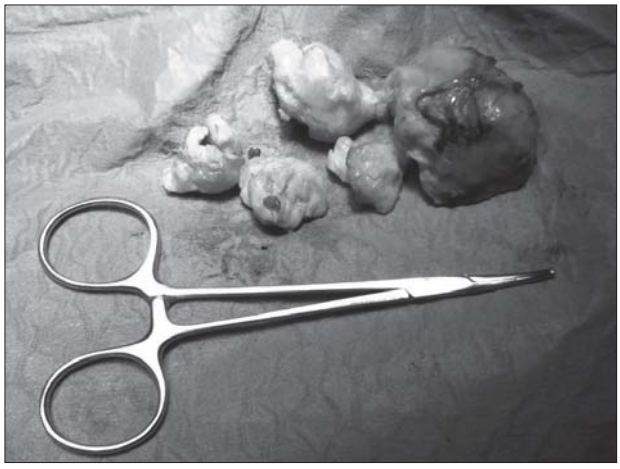

Fig. 1 - Retroperitoneal myoma after surgical resection.

leiomyoma and retroperitoneal leiomyoma (3). Retroperitoneal myoma are thus a rare finding and the great majority, like the case of our patient, are identified in the pelvic cavity.

The pathologic basis for parasitic myoma is still unclear and two main theories have been hypothesized. The older theory suggests that parasitic myoma arise from pedunculated subserosal myoma that have separated from the uterus and have managed to implant and receive blood supply from another source. A more recent theory suggest that these parasitic myoma are "iatrogenic" in nature and result from the seeding of portions of fibroids left in the abdominal cavity during morcellation at the time of myomectomy or hysterectomy. Poliquin et al. reported that over 40% of patients with retroperitoneal leiomyomas have concurrent uterine leiomyoma or a past medical history of hysterectomy due to uterine leiomyoma (4). In the case of our patient she had been previously hysterectomized due to fibroids, thus constituting in our view a possible risk factor for the development of the retroperitoneal parasitic myoma.

Although ultrasound is an excellent screening tool in these cases, magnetic resonance imaging is the most reliable imaging technique for the evaluation of retroperitoneal masses.

Conclusion

Parasitic myoma are a rare finding in clinical practice and thus constitute a diagnostic and therapeutic challenge. These benign tumors sometimes mimic adnexal masses and clinicians should be familiar with extrauterine presentations of leiomyoma. This entity should thus be included in the differential diagnosis of pelvic masses, especially in patients with concomitant uterine myoma or a previous history of myomectomy or hysterectomy due to fibroids.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Does age influence in the future of the patients with ASCUS

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Background

Cervical cancer is one of the most common neoplasic deseases affecting women with a combined worldwide incidence exceeded only breast and colonrectal cancer. The etiologic relationship of HPV infections to cervical cancer as well as to cancers at several other sites, is firmly established. Low grade lesions of the cervix reflect the pathologic effects of infection with HPV. Although about 100 HPV viral types have been identified, only 30 are known to infect anogenital tract. HPV have been divided into low risk and high risk types, based on their association with high grade squamous intraepithelial lesions and invasive cervical cancer. Although several of the low risk HPV types (most commonly types 6 and 11) are frequently found in LSIL. It is important to note that high risk HPV types, including types 16, 18, 31, 33, 35, 45, 56 are also found in ASCUS–Pap smears.

Objective

To evaluate the age as a risk factor in patients with ASCUS-Pap smears and how does it influence in the progression to precancerous lesions. The estimation is done by correlating the ASCUS citologic diagnosis with histologic biopsy.

Methods

This is a cross sectional study of patients refered to colposcopy in Regional Hospital of Durres, Albania, during the period 2009-2011. We selected the patients with the diagnose of ASCUS-Pap smears and we applied Hybrid Capture 2 (Quiagen) technique, for evaluation of High Risk HPV DNA (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).
A 12 months interval was specified in order to obtain the most valid correlation between the citologic and histologic diagnosis.
We analised patients in two groups: 30 years old and younger and older than 30 years old.

Result

We applied Pap test in 241 patient. A total of 43 (17.8%) patients were diagnosed with ASCUS according to the criteria of the Bethesta system.
From those; 26 (60.4%) patients were 30 years old and younger and 17 (39.5%) patients were older than 30 years old.
The patient's age ranged from 17-30 year old with a mean age of 23 year for the first group and 31-55 year old with a mean age of 43 year for the second group.
The occurence of High Risk HPV DNA infections in patients with ASCUS was 34.6% (9 cases) in patients 30 years old and younger and 23.5% (4 cases) in patients older than 30.
There is a significant difference in occurence on identifying the high risk HPV DNA among the women 30 years old and younger than women older than 30, with ASCUS diagnose; d=0.11, CI95%]0.2-0.2[, $p<0.01$.
The high risk HPV DNA is significantly found more often among female 30 years old and younger rather than female older than 30 years old.
We performed biopsy 12 months later, according to

the fact that histologic diagnosis is considered to be the gold standart.
In patients older than 30 years old, 75% of cases (3 patients) with positive High Risk HPV DNA test, progressed to precancerous lesions: 1 patient (25%) in CIN1 and 2 patients (50%) in CIN2.
In patients 30 years old and younger, 33.3% of cases (3 patients) progressed in precancerous lesiones: 2 patients (22.2%) in CIN1 and 1 patient (11.1%) in CIN2 (Table 1).
Meanwhile, difference in propotion on progression to the precancerous lesions between patients of age older than 30 and patients 30 years old and younger, is d=0.42 with CI 95%]0.14-0.8[, p<0.05.
There is a significant difference between the patients older than 30 years and 30 years old and younger, diagnosed with ASCUS and high risk HPV DNA, regarding the progression to precancerous lesions.

Conclusion

From our study we concluded that the prevalence of High Risk DNA HPV (in cases with ASCUS Pap smears) is higher in patients 30 years old and younger than in patients older than 30 years old. But the progression to precancerous lesions is higher in patients older than 30 years old.

Discussion

ASCUS is not a precancerous change. It is a category that indicates some cellular irritation and one that bears repeating to see if there is any change toward displasia in a later time. For some women an ASCUS results is due to changes in the cervical cells caused by HPV infection. The category of ASCUS is reserved for lesions in which a clear distinction between reactive and neoplastic cells can not be made. ASCUS diagnose is very common in young women whereas invasive squamose cell carcinoma is very rare. The risk of high grade lesions in women with ASCUS is an age related phenomen.
On the other side a risk factor identified in most studies of HPV infections is age. The maximum prevalence occured between 20 and 24 years old, followed by a decrease under age 35 years. The prevalence remained stable through age 50 years and than gradually declined again.
This study shows that the the prevalence of High risk HPV DNA in patients with ASCUS is higher in cases younger (34.5%) in comparison with cases older (23.5%).
The trends of decreasing prevalence of HPV infection with age, could be explained as a cohort effect, owing to a higher risk of HPV infection among young women today than in the past perhaps as a result of changes of sexual behavior. The prevalence rises as newly sexually active young women are exposed for the first time and than declines among older women because of decresed exposures (or acquisition of protective immunity).
According to the data of colposcopy and punch biopsy applied 12 months later, we concluded that the progression to precancerous lesions is higher in patient older than 30.
As best as can determined, the natural history of cervical carcinogenesis spams at least 1 if not 2 decades. Because of this, the likelyhood that women with an ASCUS diagnosis have HSIL on biopsy is very low if they are younger than 30 year. Because of its lower prevalence, a similar abnormality in a women older tha 30-40 years have a higher positive predictive value of finding HSIL on biopsi, especially if it is associated with HPV positive for high-rish HPV types. It is possible that lesions grows faster in this age group or that low grade lesions may not have been properly dealt with, when the patients were younger and thus progressed to higher grades.
The result of this study congruent with those of previous studies.
Investigations found high risk HPV types in as many as 86.1% of women with ASCUS.
The percentage of SIL in ASCUS smears observed from the persent and previous studies indicates that a significant porportion of ASCUS smears rapresent preneoplasic lesions. In fact in a 1998 Kaiser review, 52.6% of high grade lesion were proceed by a smear of ASCUS.

TABLE 1.

Age, yrs	ASCUS case		High risk HPV case		Biopsy case					
	nr	%	nr	%	CIN1		CIN2		Totali	
<30	26	60.5%	9	34.5%	2	22.2%	1	11.1%	3	33.3%
>30	17	39.5%	4	23.5%	1	25%	2	50%	3	75%
Totali	43	100%	13	58%	nr	%	nr	%	nr	%

A long term follow up study by Nasell et al. showed that fewer HSIL progressed to cancer in patients <25 years old than in patients >50 years old. Furthermore the progression time was significally longer for the age group <25 than for the age group 25-50 year old.

As women grow older incidence of HPV infection becomes less, but the ocurrence of cervical cancer increases.

The age, less than 30 years old seems to be a predictor factor on presence of high risk HPV ADN in patient with ASCUS, but the older age is a predictor on the progression towards the precancerous lesions. Therefore continued Pap smear screening in older woman with adjuvant high risk HPV testing can identify those woman who need further intervention for cervical cancer prevention.

All patient will high risk HPV type, including 16, 18, 31, 32, etc. should be refered for colposcopy-directed biopsy.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Risk assessment of thrombophilic states in pregnancy on the basis of indicators of biochemical markers of the first trimester

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Introduction

Successful outcome of pregnancy depends on adequate implantation, transformation of spiral artery as a result of trophoblast invasion and placentation with acquisition of adequate blood flow in the system mother-fetus-mother. Disorder of these processes is associated with genetically determined thrombi tendency when resynchronization of the processes of fibrinolysis and fibrinogenemia at implantation takes place. Under such conditions activity of protease produced by blastocyst becomes comparatively insufficient to interrupt extracellular matrix of endometrium and to invade to necessary depth. If additionally there is circulation of antiphospholipid antibodies, it makes things worse.
Taking into consideration peculiarities of physiological adaptation of hemostasis system to the pregnancy, absolute number of genetic and acquired forms of thrombophilia declares itself clinically specifically during gestational process and as it appears not only in the form of thrombosis but as well in the forms of typical obstetric complications, at that the role of various forms of thrombophilia in their pathogenesis and structure is different.
It is well known that biochemical markers of chromosomal abnormality of the I trimester (PAPP, HCG) could be the predictors of various obstetric complications (Gagnon A, Wilson RD, Audibert F et al Obstetrical complications associated with abnormal maternal serum markers analytes. J Obstet Gynaecol Can. 2008 Oct;30(10):918-49).
Lower concentrations of PAPP, HCG in the I trimester may correlate with such unfavorable lapses for pregnancy outcome as gestational toxicosis, syndrome of fetal growth retardation, premature delivery, abruption of normally situated placenta. It is worth mentioning that the latest studies show that often inherited and acquired thrombophilia is a pathogenic mechanism.
That is why we could assume that the patients with tendency for thrombophilic states at late terms of pregnancy could have accurate lower concentrations of PAPP, HCG of the I trimester.

Objective

To determine interaction between simultaneous dropping level of PAPP, HCG of the I trimester and state of hypercoagulation at late term of pregnancy.

Materials and methods

Seventy-five patients with pregnancies terminated with delivery were examined. The patients were divided into two groups. Index group was formed by 40 patients that had simultaneous inclination of PAPP, HCG lower than 0.5 MoM at gestational age of 11-13 weeks (mean value is 0.385 MoM and 0.424 Mom respectively). Control group included 35 patients with normal basal level of PAPP, HCG at the same gestational age (mean value is 1.19 MoM and 1.1 MoM respectively). The concentration of biochemical markers was determined by way of immunofluorescent method using reagent "Delphia" and analyser "Victor" (Finland). Determination of multiple of median and further assessment of risk of chromosomal abnormality was carried out with the help of programme "ALFA", UK.

The state of hemostasis was analyzed on the basis of the following markers of clotting test: prothrombin time, prothrombin ratio, INR, thrombotest, fibrinogen, APTT. The assessment of the markers mentioned above was done in 2 steps: simultaneously with of the level of PAPP, HCG and before the delivery. Thrombophilic tendency was identified on the basis of declination of some markers (rise of PTI, thrombotest, significant rise of fibrinogen (more than 6 g/l), drop of INR, shortening of PT; marker of APTT was interpreted in two ways: inclination characterized hypercoagulation, Anti-Phospholipids Syndrome was suspected at significant rise.

Results

Thrombophilic state of the I trimester in index group was found out in 4 cases (10%) vs 5 (14.29%) in control group.
Before the delivery significant hypercoagulation (which did not correspond to gestation age) was found out in index group in 10 (25%) vs control group 3 (8.57%), consequently (p<0,1).
Additional tests showed inherited thrombophilia in index group in 6 cases (15%) vs 1 (2.86%) in control group (mutation of prothrombin G20210A, FV Leiden G1691A and MTHFR C677T – isolated mutations as well as their combinations were taken into consideration); Anti-Phospholipids Syndrome in 3 (7.5%) vs 1 (2.86%) (Tab. 1).

TABLE 1.

Group	Thrombophilic state of 11-13 weeks of gestation	Thrombophilic state before the delivery	Inherited thrombophilia	Anti-Phospholipids Syndrome
Index	4 (10%)	10 (25%)	6 (15%)	3 (7,5%)
Control	5 (14.29%)	3 (8,57%)	1 (2,86%)	1 (2,86%)

Conclusions

Apparently simultaneous inclination of PAPP, HCH in the I trimester may predict thrombophilic states that lead to adverse obstetric outcomes. This is confirmed by more frequent states of hypercoagulation of the patients of index group before the delivery and more frequent detection of inherited thrombophilia in the group of such patients.
As well it is quite difficult to give answer to the question if inclination of serum content of PAPP, HCG is a compensatory reaction or if it is an evidence of breakage of compensatory mechanisms that assure favorable pregnancy outcome. However confirmation of such correlation needs further investigations of bigger amount of statistically-valid samplings.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Variability in endometrial proliferative responses to neurogenically-induced reproductive signals

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Introduction

Neurons in discrete hypothalamic nuclei i.e., the medial preoptic and ventromedial, are known to respond to vagino-cervical stimulation (vcs) resulting from manual probing or copulation (1,2). Mechanical vcs or mating stimulates similar hypothalamic neurons that regulate pulsatile secretion of gonadotropin-releasing hormone and the gonadotropins which affect deciduoma formation of decidualization, an essential for successful mammalian pregnancy, via induced ovarian steroidogenesis (3,4). These precisely applied dual reproductive signals of normal/natural copulation plus blastocyst implantation of pregnancy or artificial vcs followed by deciduogenic induction during pseudopregnancy (PPG) both elicit mechanosensory input to the brain. The two neurogenically-induced events promote synchronous endometrial proliferation/differentiation transformational responses that respectively produce a feto-maternal placenta of pregnancy, or a maternal placenta or deciduum of PPG. Additionally, any success in pregnancy is also dependent on concerted implantation, decidual and placental interactions. In fact, in our rodent model, decidual development which attains maximal growth between PPG days 9-11 in rats and mice (5), embodies either a maternal placenta under artificially-induced PPG, or a feto-maternal placenta under normal pregnancy. This progesterone-regulated decidual proliferation (6,7) and subsequent endometrial stromal cell transformation that involve regression/apoptosis (8,9), are processes that are promoted by numerous paracrine factors. Included among these factors are uterine stores of pituitary adenylate cyclase-activating polypeptide (PACAP) which promotes angiogenesis and endometrial vascularity (10) and subsequent availability of decidual metabolic substrates; decidual prolactin-related protein (dPRP) which affects decidualization by stimulating cyclic AMP (11,12); and the matrix metalloproteinases (MMPs), particularly MMP-2 and –9, enzymes that contribute to decidual invasive transformation by degrading the extracellular matrix and remodeling the decidual tissue (13,14,15).

Objective

The aim of the study was to time-relatedly assess the effects of neurogenic stimulation on: 1. decidual/placental proliferative responses triggered by copulation and blastocyst implantation during normal pregnancy and 2. on similar responses initiated by neurogenic copulomimetic vcs followed by deciduogenic stimulation under artificially-induced PPG.

Materials and methods

Female Sprague-Dawley rats (210-240g, n=5/point, under a 12L:12D photoperiod), whose estrous cycles were monitored daily by vaginal lavages and microscopic smear examination for 2-3 consecutive cycles, were randomly selected and subjected to mechanical vcs (proestrus and estrus). This was followed by surgical, laparotomized-mediated bilateral uterine trauma (PPG day 4) for PPG/decidualization induction. Other animals in groups of 2 to 3, cohabitated with a fertile male to induce pregnancy. The RT-PCR determined mRNAs of the progesterone receptor (PR), PACAP and dPRP were derived on days 3, 6, 9, 12

and 15 (1000-1100 hr) during PPG (from the decidual endometrium), or during gestation (from the gravid uterus or placenta). Deciduoma-endometrial MMP activity was detected by substrate zymography and semi-quantitated by scanning densitometric analysis during PPG.

Results

The summarized results are:

- During PPG, the levels of the PR, PACAP and dPRP transcripts increased from days 3 to 9 ($p<0.05$) and then declined.
- The anti-progesterone agent (RU-486) inhibited all mRNA expressions (dPRP, PACAP and the PR) on PPG or gestation day 9.
- There were modest elevations in MMP-2 and –9 (92 and 72 kDa) activities during deciduoma development on PPG days 3-9. Additional increases occurred during decidual regression on PPG day 15 ($p<0.05$).
- During gestation, all gravid endometrial mRNA expressions rose from days 3 to 9 ($p<0.05$) and remained elevated. Placental mRNA was consistently high from gestation days 9 to 15.

Conclusions

1. By way of the hypothalamic-hypophyseal-ovarian neuroendocrine axis, decidual/placental proliferative (endocrine and molecular) and remodeling mechanisms responded in correlated and comparable time-related profiles to artificial and normal neurogenic signals, i.e., a. copulomimetic vagino-cervical stimulation plus deciduogenic induction, or b. copulation plus blastocyst implantation. These signals induce pseudopregnancy or pregnancy in rats and mice.

2. Thus, the results indicate a potential for the artificially-induced decidual/placental rodent model to assist in the in vivo analysis of chronobiologically-related neuroendocrine communicative mechanisms between the maternal decidua and fetal cells during pregnancy.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Alternative treatment for uterine bleeding in perimenopausal women

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Background

The menorrhagia is an excessive uterine bleeding at regular intervals of time or the uterine bleeding that lasts more than 7 days and involves a blood loss greater than 80 mL (1). This symptomatology is present at 9-14% of healthy women.

Objective

The primary objective of this study is to investigate the effectiveness of progestogen therapy in achieving a reduction in menstrual blood loss in women of reproductive years with heavy menstrual bleeding.

Materials and methods

Our study includes 30 cases of menometrorrhagia at women aged between 38 and 42 years (the AUB-O group, corresponding to FIGO-PALM-COEIN classification of the abnormal uterine bleeding – polyp; adenomyosis; leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction; endometrial; iatrogenic; and not yet classified) (2). Because the quantity of blood lost at the menstruation it's hard to estimate, we used for its evaluation some individual study sheets, based on the observational criteria; the analysis relies on the obtained scores. The menorrhagia is defined through a score PBAC over 100. The patients were investigated as follows: clinical exam, cytologic exam Babes-Papanicolau, transvaginal ultrasound and biopsy fractional curettage. Using these investigations, we excluded the cervical pathology, the bleeding caused by uterine pathology - endometrial polyps or uterine fibroma. We performed hormonal analysis in days 3, 14 and 21 of a menstrual cycle, to evaluate the ovarian function.

The inclusion criteria in the study were: exclusion of the endometrial polyps and submucosal fibroids; histerometry under 10 cm; anovulatory cycles; endometrium with simple or complex hyperplasia without atypical after the endometrial sampling; endometrium measured by transvaginal ultrasound premenstrual, having a medium thickness of 1.3 cm; exclusion of haematological diseases.

The initial group was divided in 3 subgroups:

- A - 10 patients benefited of intrauterine dispositive with levonorgestrel (Mirena);
- B - 10 patients were treated with 200 mg natural progesterone intravaginal (200 mg progesterone intravaginal, between days 14 and 25 of the menstrual cycle);
- C - 10 patients received synthetic progesterone orally (lynestrenol, oral, 5 mg/day, between days 14 and 25 of the menstrual cycle).

At 6 months and 1 year a transvaginal ultrasound was effectuated to appreciate the endometrial thickness in the premenstrual stage. Analyzing individual case files using the criteria of observational study made our interpretation.

Results

After the intrauterine dispositive with Levonorgestrel (IUD-LNG) (Group A) was set, it was noted the disappearance of the menorrhagia, on an average of 5 months after they started the therapy, at 70% of pa-

tients. The main adverse effects were: breast tenderness and intermenstrual spotting in the first 3 months of treatment. The ultrasound evaluation at 6 months after the insertion of IUD-LNG shows an endometrium with a medium thickness of 6.54 mm ±1,03mm, the medium score PBAC of 63,2. At one year after starting the treatment, the PBAC score was on average 32 and the medium thickness of the endometrium was 5,84±0,52 mm.

At group B the improvement of the quality of life was present in 40% of cases. The answer to the therapy appeared on average at 4 months after starting the treatment (appreciated with PBAC score). The ultrasound evaluation at 6 months after starting the therapy with micronized progesterone showed a premenstrual endometrium of 7.55±1,2 mm and the medium PBAC score was 78. At one year after starting the therapy, the PBAC score was on average of 65,2 and the medium thickness of endometrium of 6.65±0.83 mm

At patients who received lynestrenol (group C), orally, the answer was obvious from the first month of therapy, but in this group it was registered the lowest compliance because of the adverse effects, mainly (gastrointestinal disorders, gaining weight). 40% (4 patients) of women in Group C dropped the oral hormone therapy after 3 months due to side effects and due to insignificant reduction of bleeding. The ultrasound evaluation at 6 months after starting the treatment with lynestrenol points out an endometrium of 6.9±1.34 mm and an average PBAC score of 55. At one year after beginning therapy, the PBAC score was 45,7 and the medium thickness of the endometrium 6.3±0.58 mm.

The most persistent effect regarding the decreasing of the menstrual flow was noted in the group with IUD-LNG and the fastest answer at group C of patients treated with lynestrenol oral.

The cases with AUB-O associated with simple or complex endometrial hyperplasia were evaluated to estimate the answer to the therapy with IUD-LNG vs. progesteron oral, by estimating echographically the endometrium thickness and the blood lost at menstruation at 6 months and one year after treatment. The data does not reveal any statistical relevance between the two groups neither after 6 months (6,54±0,32 versus 6,96±0,54, n=10, p >0.05), nor after one year of treatment (5,84±1,8 versus 6,3±2,5 mm, n=10, p>0.05). Instead of this, the decreasing of endometrial thickness comparing with endometrial thikness at the begining of treatment, was pointed out at 6 months after the insertion of IUD-LNG – the endometrium thickness being on average 6.54 mm ±1,03mm and after one year significantly decreased to 5.84±0,52 mm.

Discussions

The oral therapy with progesterone has the important disadvantage of a low compliancy to the treatment. The studies reveal that the therapy with IUD-LNG decreases the menstrual bleeding with 75-90%, this treatment being better than a cyclic administration of progesterone (3). The results of IUD-LNG in the treatment of AUB-O are similar to the ones obtained after the endometrium ablation with hysteroscopic guidance (4).

The abnormal uterine bleeding AUB-O is better treated with IUD-LNG, comparative with the oral therapy with progesterone, when we take into account the number of bleeding days. The thickness of the endometrium, estimated by transgavinal ultrasound, premenstrual, decreases significantly at one year after the insertion of IUD- LNG. As far as cases with anovulation associated with a simple or complex endometrium hyperplasia concern uterine mucosa thickness is not statistical relevant when compare IUD-LNG therapy with the orally administered progesterone.

Conclusions

The inside uterin or intravaginal hormonal treatment are an optimal alternative therapy of bleeding caused by anovulation cycles at women with persistent bleeding in perimenopause. The oral regimen of progestogen therapy has a positive role for obtaining in the short-term the correction of menorrhagia but in many cases it is abandoned due to significant side effects. For long-term treatment, the best choice is IUD-LNG because of its high compliance among patients and the most significant reduction of bleeding, even at slow rate.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The combined value of CA125 determination and echographic ovarian imaging, in preoperative diagnosis of benign, borderline and malignant ovarian tumors

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Introduction

The ovarian neoplasm mortality is still elevated, what endorses the approach of this subject and especially the focus to precocity of a presurgical detection, with a high probability of the ovarian tumor's histopathological type. In America, the ovarian tumor represents the third cause of mortality, after the breast and gastrointestinal cancer. In Romania, the ovarian neoplasm incidence varies between 3,5 and 5,8/100 000/year.

Objective

The objective of our study is to evaluate the efficiency of standard investigation parameters used in clinical practice to differentiate, before the surgical intervention, the benign, borderline and malignant ovarian tumors relying on echographic features and seric value of CA125.

Materials and methods

This retrospective study includes 40 cases, which were operated in the Gynecology Department of "St. Pantelimon" Clinical Hospital, Bucharest, between 2009 and 2011, based on the presurgical diagnosis of adnexal tumor. The addition criteria are: pacient age, echographic features of the ovarian mass, seric value of CA125. The abdominal/transvaginal ultrasound was performed using an echograph Voluson, 2D. All the cases were examined with a transvaginal transducer, with a frequency of 4-8 MHz. If the transvaginal ultrasound didn't detect the tumor totally, an abdominal ultrasound was performed. We analyzed the next echographic features of the ovarian tumor: dimensions (<6 cm, 6 cm, 9 cm, >10 cm), echogenity (anechogenic, hyperechogenic), unilocular/multilocular ovaries, presence of the septa, uniform/mixed echogenity, presence of papillary protuberances (solid protuberances from the cyst wall towards it's cavity measuring at least 3 mm), ± ascites. The information obtained allowed us to work with 5 study classes according to the histopathological evaluation: benign tumors, borderline tumors, malignant epithelial tumors, malignant non-epithelial tumors and rare cases of malignancy and cases with metastates (Table 1). The seric evaluation of CA125 was performed regarding all the cases of adnexal tumor. We analyzed the statistical mean of each parameter in comparison with the postoperative histopathological diagnosis of the ovarian tumor: benign, borderline and malignant, which was elaborated by the Morphopathology Department of our hospital.

Results

The operated ovarian tumors were benign in 77.5% of all cases, borderline in 5% and malignant in 17.5%. The mean value of the presurgical investigated parameters (age-years, CA125-U/mL, diameter of the tumor-cm, ±septa, vegetations), for the benign tumors are 46.58, 34.51 and 7.41. The analysed mean for borderline tumors corresponds to a value of 35, 79.58 and 7.5. As concerning the malignant tumors, the mean pertains to a value of 46, 370.83 and 12.57. The postoperative histopathological diagnose was: benign tumor for 31 cases (serous ovarian cysts, mucinous cysts,

TABLE 1 - THE HISTOPATHOLOGICAL CLASSIFICATION OF THE OVARIAN TUMORS.

Histo-pathological subtypes	Benign tumor	Histo-pathological subtypes	Borderline tumor	Histo-pathological subtypes	Invasive primary epithelial tumors	Histo-pathological subtypes	Invasive primary non-epithelial tumors	Histo-pathological subtypes	Ovarian tumors with metastates
Serous	16	Serous	1	Endometriotic adeno-carcinoma	1	Ovarian fibro-sarcoma	1	Endometrial adeno-carcinoma with clear cells, with ovarian bilateral metastates	1
Mucinous	9	Mucinous	1	Carcinoma with clear cells	1				
Endometriotic	2			Serous papillary adeno-carcinoma	1				
Adenofibroma	3			Well differentiated adeno-carcinoma	2				
Granulosa tumor	1								
Total benign tumor	**31**	**Total Borderline tumor**	**2**	**Total Invasive primary epithelial tumors**	**5**	**Total Invasive primary non-epithelial tumors**	**1**	**Total Ovarian tumors with metastates**	**1**

endometriotic cysts, cystadenofibroma, granulose tumor), borderline tumors for 2 cases (1 case-serous borderline tumor; mucinous borderline tumor), invasive primary epithelial ovarian cancer for 7 cases: endometriotic adenocarcinoma, carcinoma with clear cells, serous papillary adenocarcinoma, well differentiated adenocarcinoma, another nonepithelial cases: ovarian fibrosarcoma.

The echographic features described in documents point out data that differentiates a malignant tumor from a benign one (Table 2). The analysis of these aspects shows that the malignant tumors are defined by the presence of a solid echodense tissue inside the tumor (71.42%), intense vascularization and the presence of the ascites. The ovarian tumors that were proved to be borderline and the well-differentiated malignant epithelial ovarian tumors have intracystic papillary protuberances.

Discussions

The results of multicenter studies conducted within International Ovarian Tumor Analysis study (IOTA) and published in Ultrasound Obstet Gynecol 2000;16:500-5 and J Clin Oncol 2005; 23:8794-801 prove that the distinction between benign and malignant adnexal tumors is possible preoperatively even under the subjective interpretation of ultrasound images. The mentioned works estimate that the sensitivity and specificity in relation to the diagnosis of ovarian tumor malignancy is 88% and 96%. This is due to the differences between malignant and benign ultrasound images. Ultrasound characteristics contained in the documents reveal aspects that differentiate benign from malignant tumors.

The studies emphasize the significance of ovarian cancer screening based on determining the value of CA125 and transvaginal ultrasound. Thus, the study

TABLE 2 - ULTRASOUND FEATURES OF THE OVARIAN TUMORS.

Ultrasound characteristics	Benign		Borderline		Malign	
	Number	%	Number	%	Number	%
Dimensions						
<= 6 cm	0	0	1	50	0	0
6-9 cm	28	90.3	0	0	1	14.28
>10 cm	3	9.67	1	50	6	85.71
Chysts						
Unilocular	26	83.87	1	50	1	14.28
Multilocular	5	16.12	1	50	6	85.71
Irregular contour of the tumor walls	0	0	1	50	6	85.71
Intratumoral septa	11	35.48	2	100	6	85.71
Echogenicity						
Anechogen	26	83.87	0	0	0	0
Hyperechogen	4	12.9	0	0	5	71.42
Mixt	1	3.22	2	100	2	28.57
Papilliform images						
Missing	31	100	0	0	0	0
<3 mm	0	0	0	0	1	14.28
>3 mm	0	0	2	100	6	85.71
Vascularisation	Low		Medium		High	
Ascites	0	0	0	0	5	71.42

Prostate, Lung, Colorectal and Ovarian (PLCO) Trial conducted on a total of 39115 patients revealed that transvaginal ultrasound has been shown to be abnormal in 1338 (4.7%) of cases and abnormal CA-125 have been identified in 402 (1.4%) of cases. PLCO trial results show that the positive predictive value for invasive cancer is 3.7% for CA-125, 1% for transvaginal ultrasound and 23.5% for both tests (1). UK Collaborative Trial of Ovarian Cancer Screening, that enrolled between the years 2001-2005 a total of 50640 patients using a screening algorithm based on transvaginal ultrasound and CA125 determination, shows that the sensitivity of both screening methods is similar in primary ovarian and fallopian cancer, specificity being greater for multimodal screening (specificity 99.8% and 98.2% respectively, p <0.0001) (2).

The Risk of Malignancy Index - RMI estimates the risk for ovarian cancer at patients presenting adnexal tumor using CA-125 value, menopausal status and ultrasound characteristics of tumor formation (multilocular cyst, solid mass, ascites, bilateral lesions, metastases). Resulting values assign the patient to a low, moderate or high-risk group for ovarian cancer. The Risk of Ovarian Malignancy Algorithm (ROMA score) uses HE4 marker (Human epididymis protein 4) and CA-125 to classify in low or high-risk groups both pre-menopausal and post-menopause women. In different studies this score showed a specificity and sensitivity larger than RMI.

The preoperative CA-125 level is a good indicator for optimal cytoreductive surgery; the threshold is 500U/mL, these patients also being candidates for adjuvant chemotherapy (3).

Conclusions

The highest risk of malignancy is present at perimenopausal women for whom the highest average values both for the serum CA125 (370.83 U/ml) and for the size of adnexal tumor mass (12.57 cm respectively) were found. Serum CA125 is not suggestive for the diagnosis of borderline tumors in contrast with ultrasound changes observed in these cases. The parameters used in our clinic as preoperative investigation permit a relatively accurate estimate of the histopathological nature of ovarian tumors without any additional expensive investigations (CT, MRI).

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Amenorrhea and BMI as independent determinants of patient satisfaction in LNG-IUD users: cross sectional study in a Central European district

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Introduction

Beside oral contraceptives, IUDs are one of the most common methods of contraception in industrialized world. In addition to copper releasing intrauterine devices, Mirena®, a levonorgestrel releasing intrauterine system has been available in Central Europe since 1997 (1). According to international studies, most users of the LNG-IUD are thoroughly satisfied with it, which often depends on several factors (1,2).
Since there already exist data from international or urban areas, it seemed interesting to evaluate the situation in an Austrian region. The main effort of the study was to point out the degree of user satisfaction in women using the LNG-IUD, and to find influencing factors with special consideration of bleeding patterns and body mass index. Due to that fact, that amenorrhea is - according to other studies - not always considered to be favored in women (3,4) we checked the degree of satisfaction only concerning bleeding patterns, as well the general user satisfaction in dependence of bleeding behaviour. Furthermore factors, influencing bleeding patterns were investigated.

Materials and methods

Sample size
In this cross sectional study, data from 1825 women from 18-60 years, who visited the gynecological offices in the Austrian industrialized region during 6 months in the year 2007, was collected. All participating women received a questionnaire, concerning 26 questions, of which one question was used to allocate every women into one of three subcategories, i.e. current -, former -, or not user of Mirena. This was necessary to determine women, who had been users of Mirena but had removed it before the time of questioning, so that we were able to avoid a selectional bias. Questionnaires were filled out voluntarily and the participants gave informed consent. The local office of the research ethics comitee decided that a vote was not necessary.

Questionnaire
To evaluate the quality of the questionnaire used, a pre – test was started a few weeks ago, to have the chance to upgrade questions, which were possibly ambiguos for the patients asked. The common part of the questionnaire covered demographic topics such as age, education, number of children, body weight and height. The special part, which was only filled out from Mirena users, covered topics like changing of bleeding patterns, general user satisfaction, satisfaction concerning bledding patterns, menstrual pain, breast tenderness and skin imperfections. The purpose for using Mirena (medical reason, only for contraception, contraception and medical reason) was evaluated, too, as well as the person recommending Mirena (doctor, family member or own information).

Statistical analyses
For statistical analysis, descriptive methods and statistical tests were used. To determine differences in satisfaction dependending bleeding patterns, χ^2 tests for nominal variables and cross tabulations were done. If test criteria for the χ^2 test could not be reached, exact tests like Monte Carlo or Fisher's exact test were used. To check median values of metric variables such as body – mass – index or duration of use in context to satisfaction groups, t – test and ANOVA (analysis of variance) were conducted.

Results

Total overview and demographic characteristics
From a total number of 2000 questionnaries, 1929 were returned and 1825 could be used for computer based analysis. From all 1825 questionnaires, 415 were filled out from women who had experiences with Mirena. Table 1 gives an overview about the allocation into the categories of use.

TABLE 1 - TOTAL OVERVIEW ABOUT THE ALLOCATION INTO THE CATEGORIES OF USE.

Women using LNG-IUD: Overview		
	n	%
Current user	313	17,2
Former user	102	5,6
Never using LNG-IUD	1250	68,5
Missing values	160	8,8
TOTAL	1825	100

The median age of women, who actually carried or had used a Mirena coil, was 39,2±7,1 years and was significantly higher than in women who had no experiences with a LNG-IUD (35,4±10,9 years). 319 (77,1%) of all women, who had experiences with Mirena were married, whereas women without experiences were married in 702 (49,9%) of the cases (significant with p=0,000). In 184 (45,7%) of the cases Mirena was only used for contraception and 146 (36,2%) of all carried the LNG-IUD for contraception as well as for medical reasons (e.g. heavy bleeding). 73 (18,1%) of the women tested were not satisfied with the previous method of contraception.

Satisfaction rates and bleeding patterns
In general, 266 (65,7%) of all women asked were "very satisfied" with the LNG-IUD, 83 (20,5%) were "quite satisfied", 18 (4,4%) were "moderate satisfied" and 19 (4,7%) in each case were "less satisfied" or "really not satisfied". Concerning bleeding patterns, 295 (74,1%) were "very satisfied", with only 23 (5,8%) stating "really not satisfied". The percentage of "very satisfied" women concerning bledding patterns varied from 203 (91,0%) in women with amenorrhea to 2 (9,5%) in women with hypermenorrhea. 80 (60,2%) women of all cases with hypomenorrhea were "very satisfied" with their bleeding patterns. Amenorrhea occured in 231 women (56,9%) and was the most favoured bleeding pattern of all. In contrast to this, 13 (61,9%) of all women with hypermenorrhea were "really not satisfied" with their bleeding quality.

Table 2 gives a total overview of bleeding patterns after Mirena insertion, whereas Figure 1 shows the associated satisfaction rates:

TABLE 2 - BLEEDING PATTERNS AFTER MIRENA INSERTION – TOTAL OVERVIEW.

Bleeding patterns after LNG-IUD insertion	n	% total	valid %
Decreased blood loss	137	33,0	33,7
Increased blood loss	21	5,1	5,2
Amenorrhea	231	55,7	56,9
No changes	17	4,1	4,2
Valid total	406	97,8	100,0
Missing values	9	2,2	
Total, n	415	100,0	

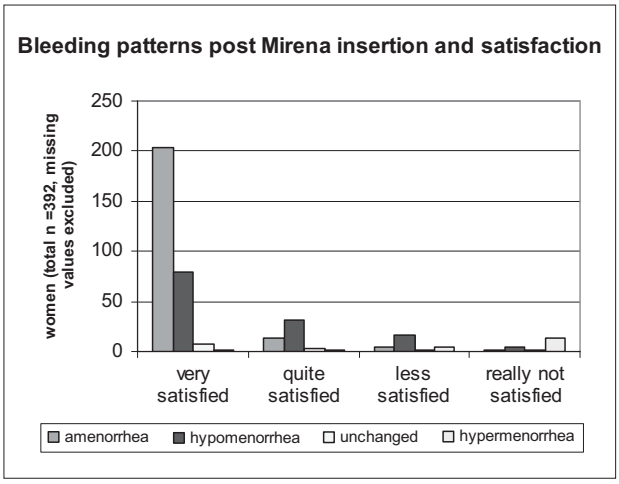

Fig. 1 - Satisfaction rates in dependance of bleeding patterns.

178 (78,4%) of all amenorrhoeic women were also "very satisfied" with Mirena in general. 73 (54,1%) women of all, who had a lighter period after Mirena insertion, were "very satisfied", and in 42 cases (31,1%) "quite satisfied". While 178 (67,9%) of the "very satisfied" women for general satisfaction were amenorrhoeic, the main part with of all "really not satisfied" women were women with hypermenorrhea (38,9% =7). The latter were only represented for 1,1% (=3) in the group of "very satisfied" women. All these differences were significant (α=0,05, p<0,001).

Bleeding patterns and duration of LNG-IUD use
In our analysis, there was a negative correlation between duration of use and bleeding patterns (r=-0,271). As shown in Figure 2, the longer the usage of Mirena, the lighter was the menstrual blood loss. While the median use of Mirena in amenorrhoeic women was 5,1±2,4

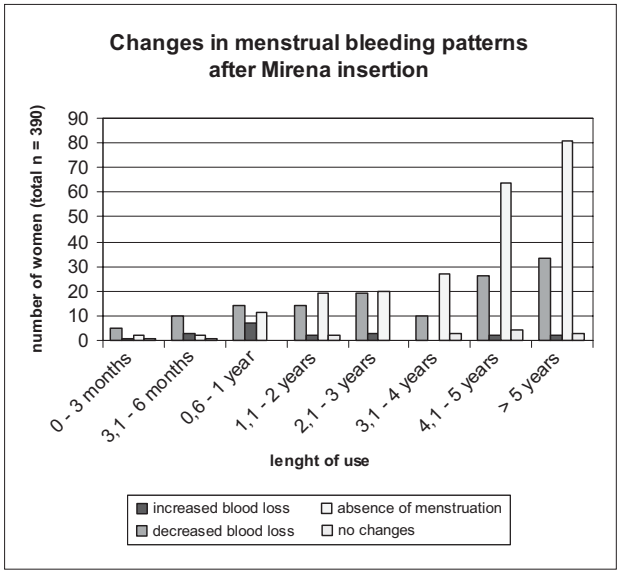

Fig. 2 - Time of usage and changes in bleeding patterns.

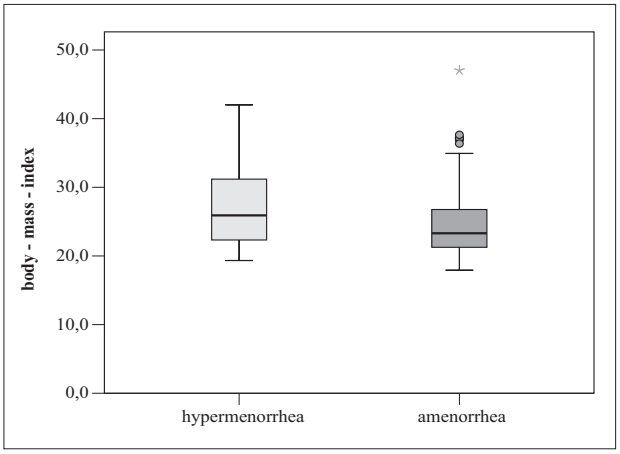

Fig. 3 - Boxplots - Bleeding patterns and BMI in LNG-IUD users.

years, the median use in women with hypermenorrhea was 2,1±2,1 years. These differences were significant with p<0,001. A more detailled analysis showed, that hypermenorrhea mostly occured after 6-12 months duration of use, but not after 36 months.

There was a significant correlation between duration of use and general satisfaction rates: the longer the usage of Mirena, the higher the satisfaction rates (r=-0,330). "Very satisfied" women used Mirena about 5,1±2,7 years, while "moderate satisfied" women had used Mirena for about 2,4±1,7 years. "Really not satisfied" user carried Mirena about 1,8±1,7 years. These differences also were significant.

BMI and satisfaction

Amenorrhea especially occurred in women with lower BMI. While the menstrual blood loss in women with a mean BMI of 27,6±6,5 kg/m^2 was subjectively described as increasing, women with a mean BMI of 24,4±4,4 kg/m^2 described amenorrhea. These differences were significant (α = 0,05 and p=0,018) Figure 3 shows the BMI differences between women with hypermenorrhea and amenorrhea.

Women who described their bleeding patterns as unchanged since using the LNG-IUD had a mean BMI of 25,9±3,5 kg/m^2. As amenorrhea was mostly considered to be a positive change and frequently found in women with a lower BMI, we also tested if there was an association between satisfaction rate and BMI. Between general satisfaction rates and BMI there proved to be no association, but between satisfaction rates concerning bleeding patterns and BMI an association was found: While "very satisfied" women had a mean BMI of 24,3±4,4 kg/m^2, "less satisfied" women had a BMI of 25,1±5,1 kg/m^2, and "really not satisfied" women of 26,2±5,4 kg/m^2 (not signficant with p = 0,268-0,976 in the particular group comparisons).

Bleeding patterns in dependance to different body – mass – index and duration of use

As described at 3.3 and 3.4, amenorrhea occured both, in women with lower BMI and in women with long duration of use. Therefore evaluating bleeding patterns by considering the categories of BMI and categories of lenght of use simultaneously made sense: For this purpose we took the widely used BMI – categories "underweight" for BMI <18,5 kg/m^2, "normal weight" for BMI 18,5-24,9 kg/m^2, "overweight" for 25,0-29,9 kg/m^2, obese class I for BMI 30,0-39,9 kg/m^2 and II for BMI >40,0kg/m^2 (5).

While 2 (50%) of the normally weighted women got amenorrhoeic in the first three months of use, 0% of overweighted and obese women did (not significant). After 6 months-1,0 year of use, 9 (50%) of all normally weighted women described amenorrhea, whereas only 1 (11,1%) woman with overweight and 1 (25,0%) with obesity class I did. After 1,1-2,0 years of use, 8 (42,1%) of all normally weighted women described amenorrhea, but 9 (60,0%) of overweighted women and 2 (100%) of obese women class I did. 18 (28,6%) of all oveweighted women and 5 (23,8%) of all obese class I women had amenorrhea after 4,1-5 years of use. So in general, women with higher BMI also got amenorrhoeic when using the LNG-IUD, but showed a longer time of LNG-IUD use than normally weighted women. Figure 4 gives a total overview of all amenorrhoeic women after LNG-IUD insertion considering the body mass index and lenght of IUD use.

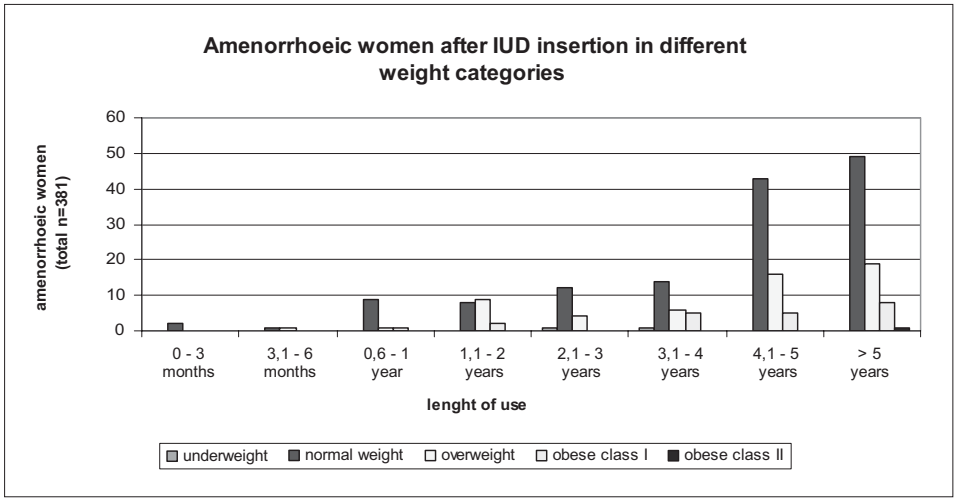

Fig. 4 - Overview of all amenorrhoeic women after LNG-IUD insertion, considering BMI and duration of use simultaneously.

Menstrual pain and satisfaction

Menstrual like pain disappeared in 138 (37,3%) cases after Mirena insertion, and was lightened in 71 (19,2%) women. 33 (8.9%) women had no changes in menstrual pain and 9 (2,4%) suffered from increased menstrual like pain 2,4% (=9).

There was a significant correlation (r=0,605) between bleeding patterns and menstrual pain. In amenorrhoeic women menstrual like pain disappeared in 106 (54,5%) of the studied cases, and 29 (21,6%) of women with lighter period also reported less menstrual pain. 4 (20,0%) of women with heavier menstrual bleeding after LNG-IUD insertion also reported increased menstrual like pain. All differences were significant with p<0,001. 132 (95,7%) of the participantes, who had no pain at all, were "very satisfied" with their situation. 43 (61,4%) of all women with lighter menstrual pain were "very satisfied", whereas 7 (63,6%) with no changes of menstrual pain were "really not satisfied". The results concerning general satisfaction were similar: While 108 (79,4%) of all women with reduced pain were "very satisfied", most of the not satisfied women had no changes in menstrual pain. All these differences were significant with p<0,001.

Intention for using the LNG-IUD and satisfaction

From a total number of 406 women (9 missing), 184 (45,3%) used the LNG-IUD only for contraception. 146 (36,0%) used it for contraception and had at least one medical reason (e.g. lighter bleeding). 73 (18,0%) used the coil for contraception and were unsatisfied with the previous method of contraception. 3 (0,7%) women declared other reasons for using the IUD.

110 (62,1%) of all women who used the LNG-IUD only for contraception were very satisfied in general; (13 missing values from total excluded). 103 (71,0%) of women who used the LNG-IUD for contraception and a medical reason were very satisfied. (1 missing). 49 (68,1%) women who used the coil for contraception and were unsatisfied with the previous contraceptive method, were very satisfied it. (1 missing from total). Comparing all groups, most "really not satisfied" women with n=10 (58,8%) were found in the group of women, who used the coil only for contraception. Table 3 gives an overview.

Continuation rates:

58 (56,9%) of a total of 102 former Mirena users removed the IUD within 4,5 years and 41 (40,2%) after

TABLE 3 - INTENTION FOR USING LNG-IUD AND SATISFACTION RATES, OVERVIEW. MISSING VALUES EXCLUDED.

Intention for using LNG-IUD	Grade of Satisfaction					
	very	quite	moderate	not	really not	Total
Only for contraception	110	34	10	13	10	177
For contraception and medical reason	103	31	3	5	3	145
Contraception + unsatisfied with previous method	49	17	3	1	2	72
Other	1	0	0	0	2	3
TOTAL	263	82	16	19	17	397*

* missing values caused by cross tab-analysis, excluded.

5 years of use, 3 (2,9%) values missing. So we had a discontinuation rate of 14,0% for our total number of 415 women.
The most frequently declared reasons for discontinuation in multiple answer system were "unsatisfied with bleeding" and "wish for children" with 14 (15,2%) women in each case. 11 (12,0%) participants reported pain, followed by weight increase from 8 (8,7%) women, expulsion and skin imperfections in 7 (7,6%) cases respectively. The wish for children excluded, the most important reason for discontinuation was "unsatisfied with bleeding" with 12,1% (=7). 11 (55,0%) of the women with increased menstrual blood loss removed the LNG-IUD prematurely, whereas only 24 (10,5%) with amenorrhea and 16 (11,7%) with hypomenorrhea did.
3 (33,3%) of all women with increased menstrual pain stopped using the IUD. With a percentage of 37,5% (=12) the discontinuation rate was a little bit higher in women with no changes in menstrual pain. Similar to the results concerning bleeding patterns, also the discontinuation rate concerning menstrual pain were lowest in women without any pain (6,6% = 9) or rather decreased menstrual pain (14,1% = 10). According to Fisher's exact test, all these differences were significant with p<0,001.

Discussion

International comparisons
According to international data, most Mirena users are really satisfied with the LNG-IUD (6). Backman et al showed, that 74% of Mirena users were "very" or "quite" satisfied (2), which is comparable with our results of 65,7% "very" – and 20,5% "quite" satisfied women. Of the 313 current users in our study, 95,1% were "very" or "quite" satisfied with Mirena, which is nearly as high as the percentage of satisfied women described by Backman et al 2002 with 91% (2). Changes in bleeding patterns seem to be an important influencing factor on satisfaction: Women with amenorrhea were more often "very satisfied" as well for general satisfaction as for satisfaction rates only considering menstrual changes, than women with increased blood loss. The fact, that 78,4% of all amenorrhoeic women, but only 15,8% of women with hypermenorrhea were "very satisfied" leads to the aspect, that nowadays absence of menorrhea is favoured. However, conflictive opinions were found (3). According to Glasier et al. 2003, amenorrhea was not accepted in 1970/80, while in 2000/01 women of well developed countries prefer amenorrhea or reduced menstrual blood loss (4). Baldaszti et al. described in 2003, that amenorrhea was seen as a positive change in 81% of Mirena users (1), which is similar to the results of a study from 2006, where decreased blood loss was also favoured by women (7). In our study, which was conducted in 2007, women with amenorrhea or decreased blood loss were more often "very satisfied" than women with other bleeding patterns, which leads to the conclusion, that the "modern woman" seems to prefer lighter periods or absence of menorrhea. In contrast to this, amenorrhea was described as a negative change in a study from 2007, when 40% of all LNG-IUD users declared amenorrhea as an unwanted effect (8). However, this result has to be seen very critically, because amenorrhea was automatically countered as a negative change, independent of the women's real attitude towards it. In our analyses, amenorrhea occurred in 56,9% of Mirena users, and lighter periods in 33,7%, which is well comparable with other studies, where amenorrhea and hypomenorrhea were described in 56-88% (1,6,9).
According to international data, a decreased menstrual blood loss could be reached in women with hypermenorrhea in 79-97%, when treated with the LNG-IUD (3).
In our results, the usage of Mirena was longer in women with amenorrhea or hypomenorrhea, than in women with hypermenorrhea, which was statistically measured with a correlation of r=-0,271 and p<0,0001. That showed, that longer usage lead to a lighter menstrual blood loss. The median value of use was 5,1 years in women with amenorrhea, and 2,1 years in women with hypermenorrhea. The latter was more often described after 6-12 months of use, but not after 36 months. Kriplani et al. also described a decreased menstrual blood loss more often in women with long-time use of the LNG-IUD, than in women with shorter time of use (10). Similar to that were the results of Backman, who showed in 2004, that the menstrual blood loss decreased continuosly with the lenght of using LNG-IUD (6). Although the LNG-IUD is admitted for 5 years of use, our analyses also show women with lenght of use more than 5 years: Reasons for that are, that women have a second coil, that they forgot to remove or that the IUD was used for off label use (e.q. praemenopausal menstrual disorders). Absence of menorrhea is well described as a result of the local endometrial receptor interaction with the LNG, but is not mainly caused by hormonal plasma levels (6). Furthermore, the individual bleeding behaviour was described as independent from the ovarial function (11). In our study we found an association between length of use and amenorrhea, which can be of course explained with the local endometrial interaction, but we also found an association between individual BMI and bleeding patterns. However, our results showed, that

women with hypermenorrhea had a significantly higher BMI than women with no bleeding changes (~27,6 kg/m^2 vs ~ 25,9, kg/m^2). Women with amenorrhea had a BMI of ~ 24,4 kg/m^2, which was the lowest value of all group – comparisons. Therefore we conclude, that in our study women with a higher BMI were more likely to have heavier periods than women with a lower one. The BMI difference of 3,2 kg/m^2 between women with hypermenorrhea and women with amenorrhea doesn't seem to be excessively high, but means, that a difference of 10kg weight in 170cm high persons is enough, to influence the bleeding pattern. As lenght of use and BMI seem to be necessery influencing factors for bleeding patterns in each case, and interaction between them is not clear, bleeding patterns afte all normally weighted women had amenorrhea in the first three months of use, 0% of overweighted and obese class I women had. In contrast to this, 18 (28,6%) of all oveweighted women and 5 (23,8%) of all obese class I women had amenorrhea after 4,1-5 years of use. So it seems, that women with higher BMI get later amenorrhoeic than normally weighted women.

However, according to earlier studies, LNG-IUD users with a high BMI are not likely to suffer more often from unwanted pregnancies than other women (6). In contrast to this, the rate of contraceptive failures can be higher in women with a high BMI when using the hormonal patch (12).

In our study we found no association between general satisfaction rate and BMI, but between satisfaction rate concerning bleeding patterns and BMI (not significant): Concerning bleeding patterns, "very satisfied" women had a median BMI of ~24,3 kg/m^2, while "really not satisfied" women had a median value of ~ 26,2 kg/m^2, which would be a difference in weight of 6 kg for 170cm high persons. The percentage of "very satisfied" women concerning bleeding patterns varied from 91,0% for women with amenorrhea to 9,5% for women with hypermenorrhea. For general satisfaction rate, women with amenorrhea also were more satisfied than women with hypermenorrhea. Previous studies showed that menstrual pain as well as bleeding disorders are common reasons for discontinuation (6), and that user satisfaction increased with decrease of menstrual pain (1). In our study women with increased menstrual blood loss removed the IUD for a higher percentage prematurely than women with lighter or no periods (55,0% vs 11,7% 10,5%). Backman et al. showed in 2002 similarly, that women with hypo- or amenorrhea, had a lower discontinuation risk than women with heavy menstrual bleeding (13). Concerning dysmenorrhea, 33,3% of women with increased menstrual pain discontinued LNG-IUD use, while 6,6% of women without any pain did. The highest discontinuation rate with 37,5% was observed in women with no changes in menstrual pain since using Mirena. We conclude that menstrual bleeding patterns and pain are important factors that don't only influence patient satisfaction, but also decisions for or against discontinuation.

According to Backman et al. (2002), advanced information, especially concerning bleeding patterns, can improve user satisfaction (2). If we take Backman's finding into account and note that women with a higher BMI are more likely to suffer from heavier menstrual bleeding, women should given individual information about Mirena and it's causing bleeding changes, depending on BMI and body composition.

Limitation(s) of the study

Because of the retrospective study design, we can of course not avoid recall bias or prove the correctness of women's answers. But satisfaction is anyway a very subjective sensation. As our sample size of 415 women was divided into small sub categories for analysing, the statistical power of our results is of course limited. We mostly used normally scaled – or ordinally scaled values, therefore some statistical tests were not feasible, and often statistical tests of lower level had to be done. Therefore often descriptive statistic was used. However, infomative value is still high, because there don't exist any studies, which deal in detail with the same topic. We are not aware of any papers, that remind the body mass index when evaluating patient's satisfaction or bleeding patterns in women with the LNG-IUD. But this aspect is of course relevant for everyday's working life, because women with higher BMI could given another information about bleeding changes by the use of IUD than women with lower BMI.

Even though our sample size was of only 415 women, the dimensions of our BMI groups were similar to that of the Central European average: According to the Austrian average 64,0% of the 30-44 years old Austrian women were in the BMI group of 18,5-24,9 kg/m^2, whereas in our study 63,6% of women at the same age were. To obesity class I, the public Austrian statistic counters 5,8% of all 20-29 years old women, and 9,4% of all 30-44 years old women. Similarly to that, 6,5% of all women 20-29 years old in our study were in obesity group I, and 10,2% of all women aged between 30-44 years. Despite of our small sample size, the study seems to reflect well the average Austrian women.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Biomarkers of angiogenesis and inflammatory response in peritoneal fluid of women with endometriosis

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Introduction

Endometriosis is one of the most common gynecological conditions. The prevalence of endometriosis is really unknown. The disease is found in about 0,5-5% women of reproductive age, mostly in those (25-40%) with fertility problems. Some authors reported a more frequent occurrence of the disease. The most common symptom for women who have endometriosis is cyclic pelvic pain, which may also be chronic in nature and that may fluctuate with age. Angiogenesis and inflammatory response play a pivotal role in the pathogenesis of endometriosis and implantation of endometrial tissue outside the uterus. Angiogenesis is a process partially regulated by vascular endothelial growth factor (VEGF) – a signalling protein family which takes part in creating new blood vessels. The severity of endometriosis symptoms is a common cause of work absence. This fact and the necessity of invasive procedures: laparoscopy or open surgery conducted to establish the diagnosis, affect social costs associated with endometriosis.

There is no treatment option that could be efficient enough in all types of endometriosis. Danazol – known from early 80-ties of the XX century is a therapeutic option that is cheap, easy available and acceptable by patients. The in vivo influence of danazol on angiogenesis and inflammatory response is not well documented. Nowadays the main goal is to find a marker or a group of biomarkers for endometriosis which could also be helpful in monitoring the treatment. Having such a marker would avoid second-look laparoscopies for endometriosis and lower the costs associated with the disease. Our research on peritoneal fluid concentration of some markers typical for angiogenesis and inflammatory response is a part of a major experiment which goal is to find a non-invasive procedure to diagnose endometriosis. Besides we evaluate VEGF (vascular endothelial growth factor), Ca-125, IL-1β and CRP concentration in plasma of patients with endometriosis and after treatment this disease with danazol.

Aim

The main goal of this study was to evaluate Ca-125, VEGF, IL-1β and CRP concentrations in peritoneal fluid (PF) in patients with endometriosis

Materials and methods

This case-control study included 103 Caucasian women in reproductive age admitted to the First Department of Gynaecology and Obstetrics Medical University of Łódź (Poland) for diagnostic or therapeutic laparoscopy. The patients underwent laparoscopic surgery for infertility, pelvic pain or endometriosis suspicion between February and November 2010. Those with laparoscopically confirmed endometriosis who fulfilled inclusion criteria were qualified to the study group, patients without this condition served as a comparative group for the statistical analysis. Women with any conditions known as influencing Ca-125, VEGF or CRP concentration and with ovarian malignancy established by intraoperative histopathologic examination as well as women in the luteal phase of the cycle were excluded from the study. The study protocol was approved by the Ethics Committee of the Medical University of Łódź . All subjects included in the study signed informed consent. Dur-

ing the laparoscopy a peritoneal fluid specimen and an endometrium biopsy was taken. The biopsy of the endometrium was histopathologicaly examined to establish a phase of the menstrual cycle. The analysis of concentrations of Ca-125 and CRP was conducted by radioimmunoenzymatic methods. VEGF and IL-1β levels were established by ELISA strictly according to the manufacturer's instructions. The presence and extent of endometriosis were carefully assessed, in accordance with the rASRM 1996 (The Revised American Society for Reproductive Medicine) classification of endometriosis. rASRM classification was adopted for statistical purposes: mild endometriosis = EEC of I and II stage, severe endometriosis = EEC of III and IV stage. Statistical comparisons were performed using Statistica 7.0 (Stat Soft Polska). Regardless of the statistical test, only P-value ≤ 0.05 was considered significant.

Results

Endometriosis was confirmed laparoscopically in 68,9% of the women (n=71). The differences in age, menarche, menstruation length and duration of infertility were not statistically significant between the study and comparative group. There were statistically significant differences in the BMI and pain associated with the menstrual bleeding between groups (78,9% women with this symptom in the study group vs 43,8% in the comparative group; p<0,001).
The group with endometriosis presented significantly higher plasma concentration of Ca-125 and significantly lower levels of VEGF. IL-1β and CRP plasma concentrations were comparable between groups. There were also no differences in concentration of these two markers between subgroups with the minimal-mild and severe endometriosis.
Analysis of PF concentration of the studied markers showed, that groups varied significantly only in Ca-125 levels (controls: mean value: 1054,85; SD 1135,61, n=32 vs. mean value: 2128,04; SD 2058,95, n = 70 in study group; p=0,006) and VEGF concentrations levels (controls: mean value: 175,76, SD 116,13, n=19 vs. mean value: 354,65 SD 609,12 n=48; p=0,055). VEGF was also significantly higher in the subgroup with severe endometriosis in comparison to the group with mild endometriosis (controls: median: 235,95 Q25: 212,20 Q75: 352,50 vs median: 190,70 Q25: 129,79 Q75: 231,70 p=0,012). Its concentration in peritoneal fluid correlated well with the stage of the disease (Spearman's rank correlation coefficient R=0,35; p=0,016). The concentration of CRP (mg/l) and IL-1β (pg/ml) between the groups was not statistically significantly different.

Discussion

The role of determining Ca-125 concentration in plasma is well known in the diagnosis of endometriosis. Studies on its role in peritoneal fluid present discrepancies. The levels of Ca-125 in PF in our study are higher than those determined by other researchers. This fact is probably influenced by the use of one of the newest tests for detecting antigen OC 125 (VIDAS II). One of the most important angiogenic factors, i.e. VEGF, was selected to evaluate angiogenesis in endometriosis. The influence of VEGF on IL-1β plays a role in angiogenesis and inflammation response. It was therefore concluded that the analysis of the concentration of these markers will best characterize the mentioned processes in the women with endometriosis. Some studies confirmed the higher concentration of VEGF and Il-1β but others found no correlation between endometriosis and the concentration of mentioned biomarkers in PF. In our study the concentration of VEGF was significantly higher in women with the disease in comparison to the control group but we did not find any relation between Il-1β level in PF and the disease. Moreover, a strict adherence to the criteria for the qualification procedure (e.g. excluding the women in whom a cycle phase was not histopathologically confirmed) seems to have an influence on the correlation between the VEGF concentration and the severity of the disease.

Conclusions

Despite high prevalence of endometriosis in the studied population, the group with endometriosis was well characterized by parameters known as typical for the disease: painful menses, increased Ca-125 concentration in plasma and in peritoneal fluid, increased VEGF concentration in peritoneal fluid.
Higher VEGF values in PF confirm the role of angiogenesis in the development of endometriotic foci outside the uterus in the first cycle phase of women with endometriosis.
There is a clear correlation between the severity of the disease and VEGF concentration in PF (p=0.012).
Il-1β and CRP are not enough sensitive to distinguish between women with and without endometriosis.

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Diabetic nephropathy in pregnant women with type 1 diabetes mellitus

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Introduction

Microalbuminuria (urinary albumin excretion in the range of 30-300 mg/24 h) is an early manifestation of diabetic kidney disease. It is associated with slightly elevated blood pressure (BP) within normal range and subclinical edema due to universal vascular leakage of albumin, and it predicts overt diabetic nephropathy with persistent proteinuria and hypertension (1,2,3).
The most important problem in the pregnancy complicated by type 1 diabetes is increased perinatal morbidity associated with preterm delivery. Up to one-third of infants of mothers with type 1 diabetes are delivered preterm (4,5,6), while the prevalence of preeclampsia characterized by hypertension, proteinuria, and edema is 10-20%. Diabetic nephropathy (urinary albumin excretion >300 mg/24 h) presents at conception is a major contributor to increased perinatal morbidity and mortality (7,8). Diabetic women with high early pregnancy proteinuria of 190-499 mg/day have been reported to have an increased risk of developing preeclampsia, similar to that in women with diabetic nephropathy (9,10,11). The aim of this study was to determine the influence of microalbuminuria on fetal outcome and maternal complications in pregnant women with type 1 diabetes.

Patients and methods

In our study were enrolled 80 women with type 1 diabetes before gestation who were admitted to the Department of Obstetrical Gynecology at American Hospital, "Hygeia" Hospital, Diagnostic Center "Ikeda-Euromedica"and Diagnostic Center "Med.al" before 17 weeks of gestation with a living fetus. Women who had miscarriages (≤ 22 weeks of gestation) were excluded.
The cases were analized for urinary albumin excretion, BP, HbA_{1c}, that were measured by various methods in the respective centers. The women were categorized according to their level of urinary albumin excretion, calculated as geometric mean of two to three measurements. Normal urinary albumin excretion was defined as <30 mg/24 h, microalbuminuria was defined as urinary albumin excretion of 30-300 mg/24 h, and diabetic nephropathy was defined as urinary albumin excretion >300 mg/24h (12,13). Data regarding kidney function and BP status at baseline are summarized in the following sections.

Normal urinary albumin excretion
In 54 women without kidney involvement, geometric mean urinary albumin excretion was 7 mg/24 h (range 1-27). Two of them (2,5%) were treated with antihypertensive drugs before pregnancy (ACE inhibitors, beta blockers, and/or diuretics).

Microalbuminuria
In 18 women with microalbuminuria, geometric

mean urinary albumin excretion was 65 mg/24 h (range 14-270). Five of them were normotensive, because they were diagnosed with hypertension and treated with ACE inhibitors before pregnancy.

Diabetic nephropathy

In 8 women with diabetic nephropathy, geometric mean urinary albumin excretion was 1,115 mg/24 h (range 458-5,480). Two women had nephrotic proteinuria >3 g/24 h; the mean serum creatinine was 87 µmol/l (range 58-165), and in the first trimester, three women had a creatinine clearance <1.13 ml/s. Five women were treated with antihypertensive drugs (three with ACE inhibitors and three with beta blockers, possibly in combination with diuretics), because they were diagnosed with hypertension before pregnancy.

The women were classified according to the White classification, which is traditionally used in obstetrics to grade the severity and duration of diabetes. White class B was defined as onset of type 1 diabetes after 19 years of age and duration of diabetes <10 years. White class C was defined as onset of type 1 diabetes between 10 and 19 years of age or duration of diabetes 10-19 years. White class D was defined as onset of type 1 diabetes before 10 years of age or duration of diabetes ≥20 years or presence of retinopathy. White class R was defined as presence of proliferative retinopathy, and White class F was defined as presence of diabetic nephropathy (15). A woman was categorized as a smoker if she smoked ≥1 cigarette per day.

The women were asked to perform home blood glucose measurements at least four times daily during their pregnancy and to adjust insulin doses accordingly in order to maintain preprandial blood glucose levels between 3 and 6 mmol/l. They visited the obstetric clinics every 1 or 2 weeks during pregnancy. Labor was routinely induced at 37-40 weeks of gestation based on individual evaluation.

Single measurements of 24-h urinary albumin excretion, HbA_{1c}, and office BP were performed at least five times throughout the pregnancy (weeks 10, 14, 20, 28, and 34). Urine samples were analyzed for albumin by enzyme-linked immunosorbent assay (14) or by a turbidimetric method using the same antibodies and buffers. HbA_{1c} was analyzed by high-performance liquid chromatography (17) or by antibody immunoassay (normal range 4.1-6.4%). Office BP was measured in a sitting position with the arm at heart level after 5-10 min rest.

The diagnosis of preeclampsia in women with normal urinary albumin excretion or microalbuminuria was based on the presence of office BP >140/90 mmHg (three measurements) accompanied by proteinuria >0.3 g/24 h (two urine samples) later than 20 weeks of gestation (proteinuria of 0.3 g/24 h is equivalent to a urinary albumin excretion of 190 mg/24 h). The diagnosis of preeclampsia in women with diabetic nephropathy was based on the same findings as well as a sudden increase of ≥15% in systolic or diastolic BP (11). Pregnancy-induced hypertension without proteinuria was defined as the development of BP >140/90 mmHg (three measurements) later than 20 weeks of gestation in women who were previously normotensive and proteinuria <0.3 g/24 h. Antihypertensive treatment with methyldopa was initiated due to preeclampsia (n = 13) or if diastolic BP was higher than 95 mmHg without proteinuria (n = 11) or if proteinuria exceeded 3 g/24 h (n = 2) in the absence of hypertension. None of the women with normal urinary albumin excretion or microalbuminuria required antihypertensive treatment early in pregnancy (<20 weeks of gestation). In addition to insulin and hypertensive drugs, four women were taking levothyroxine for Hashimoto Thyroiditis, five were taking antidepressive drugs and one was taking an antiepileptic drug during the pregnancy.

Preterm delivery (<37 weeks of gestation completed) included spontaneous delivery and delivery based on obstetric indications such as uncontrolled hypertension, severe symptoms of preeclampsia, or macrosomia. Small for gestational age was defined as <10th centile for gestational age. Perinatal mortality was defined as fetal death later than 22 weeks of gestation or within 1 week after delivery. Major congenital malformations were considered those responsible for death, those causing a significant future disability, or those requiring major surgery for correction (16). During the study period, no fetuses with congenital malformations were aborted as late abortions.

Statistical analysis

Normally distributed continuous variables are given as means ± SD, and urinary albumin excretion is given as geometric mean and range. χ^2 trend test for categorical data and one-way analysis of variance linear trend test (regression) for continuous variables were applied to compare groups. Multivariate logistic regression analysis was applied to identify variables independently associated with development of preterm delivery and preeclampsia. Category of urinary albumin excretion and tertiles for HbA_{1c} (≤ 7, 7.1, 7.9, and ≥8%) were applied as independent variables in the logistic regression analysis. Probability value <0.05 (two-tailed) was considered significant.

Results

A total of 54 women (67.5%) had normal urinary albumin excretion, 18 (22.5%) had microalbuminuria, and 8 (10%) had diabetic nephropathy at baseline (Table 1). There was a trend toward longer duration of diabetes and higher BMI, HbA_{1c}, and BP with increasing category of urinary albumin excretion.

Antihypertensive therapy was given in 9 of 54 women with normoalbuminuria, 8 of 18 women with microalbuminuria, and all 8 women with diabetic nephropathy. Mean systolic blood pressure during pregnancy was 124 mmHg (range 105-151), 125 mmHg (119-138), and 138 mmHg (113-147) in women with normoalbuminuria, microalbuminuria, and diabetic nephropathy, respectively (P=0.0095). No differences in mean diastolic blood pressure or HbA_1C were detected between the groups. No women with microalbuminuria developed preeclampsia. The frequency of preterm delivery was 22% in women with normoalbuminuria and microalbuminuria, in contrast to 75% in women with diabetic nephropathy (P<0.01) where the median gestational age was 254 days. When excluding the group with diabetic nephropathy from analysis, women with microalbuminuria still had a higher prevalence of preterm delivery compared with women with normal urinary albumin excretion (P<0.05). The increased prevalence of preterm delivery was mainly due to the higher prevalence of preeclampsia in women with microalbuminuria or diabetic nephropathy. A total of 24% of the women with microalbuminuria and 60% of the women with diabetic nephropathy delivered preterm due to preeclampsia. Preterm delivery due to other causes (spontaneous or for obstetrical reasons) was comparable in the three groups.

Increased prevalence of intrauterine growth retardation was seen in women with diabetic nephropathy but not in women with microalbuminuria (Table 2). Diabetic nephropathy was also associated with increased prevalence of jaundice requiring treatment, and there was a tendency toward a higher proportion of development of tachypnea requiring assisted ventilation. Perinatal mortality and major congenital malformations were comparable in the three groups.Using multivariate logistic regression analysis, the baseline variables of urinary albumin excretion, systolic BP, HbA_{1c}, White classification, age, BMI, parity, and smoking were tested as predictors of preterm delivery. Increased category of urinary albumin excretion (P<0.01) and high HbA_{1c} at 2-6 weeks of gestation (P<0.05) were independently associated with preterm delivery.

Discussion

In our study, the presence of microalbuminuria during the pregnancy and higher HbA_{1c} at 2-6 weeks of gestation has been associated with increased risk of preterm delivery in women with type 1 diabetes mellitus. The substantially increased prevalence of preterm

TABLE 1 - BASELINE DATA ON 80 PREGNANT WOMAN WITH TYPE 1 DIABETES AND NORMAL URINARY ALBUMIN EXCRETION, MICROALBUMINURIA AND NEPHROPATHY.

	Normal urinary albumin excretion	Microalbuminuria	Nephropathy	*P* value
Patients, *n*	54	18	8	–
Age (years)	28±5	30±3	30±4	NS
Duration of diabetes (years)	10±6	17±4	18±5	<0.01
BMI (kg/m^2)	23±4	25±6	26±4	<0.05
Urinary albumin excretion (mg/24 h)	7 (1–26)	72 (18–280)	1,118 (470–5,540)	ND
HbA^{1c} at 2–6 weeks (%)	7.2±1.4	8.0±1.1	9.0±1.4	<0.001
Early systolic BP (mmHg)	124±13	125±12	138±11	<0.001
Early diastolic BP (mmHg)	67±9	70±7	79±7	<0.001
White classification				
B + C	30 (55.5)	7 (39.9)	0	ND
D + R	24 (45.5)	11 (61.1)	0	ND
F	0	0	8 (100)	ND
Nullipara	29 (55.5%)	9 (50%)	4 (50%)	NS
Smokers	14 (25.9%)	4 (33.3%)	4 (50%)	NS

Data are means ± SD, mean (range), or *n* (%).
The statistics applied are χ^2 trend test when comparing categorical data and linear trend test (regression) for one-way analysis of variance when comparing continuous data.
ND: not done.
NS: not significant.

TABLE 2 - PREGNANCY COURSE AND OUTCOME IN 80 WOMEN WITH TYPE 1 DIABETES AND NORMAL URINARY ALBUMIN EXCRETION, MICROALBUMINURIA AND NEPHROPATHY.

	Normal urinary albumin excretion	Microalbuminuria	Nephropathy	*P* value
Patients, *n*	54	18	8	–
HbA_{1c}, weeks 10–34 (%)	6.4±0.8	6.8±0.5	7.4±0.7	<0.01
Preeclampsia	4 (7.4)	7 (38.8)	5 (62.5)	<0.001
Pregnancy-induced hypertension without proteinuria	2 (3.7)	1 (5.5)	0	NS
Proteinuria >3 g/24 h	0	5 (27.7)	5 (62.5)	<0.001
Preterm delivery before week 37	2 (3.6)	2 (11.1)	4 (50)	<0.001
Preterm delivery before week 34	0	1 (5.5)	2 (25)	<0.001
Perinatal mortality	0	1 (5.5)	0	NS
Singleton small-for-gestational-age infants (<10%)	1 (1.8)	1 (5.5)	3 (37.5)	<0.001
Birth weight, singletons (g)	3,478±595	3,124±678	2,185±1042	<0.001
Major congenital malformations	1 (1.8)	1 (5.5)	1 (12.5)	NS
Tachypnea continuous positive pressure <1 h, singletons	8 (14.8)	3 (16.5)	2 (25)	NS
Jaundice requiring treatment, singletons	8 (15)	1 (5.5)	5 (62.5)	<0.01

Data are n (%) or means ± SD.
The statistics applied are χ^2 trend test when comparing categorical data and linear trend test (regression) for one-way analysis of variance when comparing continuous data.
NS: not significant.

delivery with the increasing degree of albuminuria was caused by higher prevalence of preeclampsia.

Our findings are in accordance with Combs et al. (11), who reported an increased prevalence of preeclampsia and preterm delivery in women with early pregnancy proteinuria of 190-499 mg/24 h.

A substantial number of the women with microalbuminuria increased their protein excretion to the nephrotic range during pregnancy. This is in accordance with Biesenbach et al. (19), who found that nephrotic proteinuria developed in 4 of 12 women with microalbuminuria during pregnancy. One might speculate whether antihypertensive treatment during pregnancy should be indicated with increasing albumin excretion. We chose to initiate antihypertensive treatment in normotensive women with proteinuria exceeding 3 g/24 h during pregnancy. After delivery, the albumin excretion has been shown to return to prepregnancy levels (12,17).

The prevalence of preterm delivery in our study was comparable to the literature (6), although some authors have reported a higher prevalence of women delivering at term (5,6,).

The association between high levels of HbA_{1c} and development of preeclampsia has been described earlier in type 1 diabetes (9,18). In our study we have noticed a correlation between elevated HbA_{1c} at 2-6 weeks of gestation and increased prevalence of preterm delivery. The presence of hypertension is of importance for the prediction of preeclampsia (19,20).

In women with microalbuminuria or diabetic nephropathy, development of preeclampsia was the most important single cause of preterm delivery. In women with normal urinary albumin excretion, we also found a surprisingly high prevalence of preterm delivery associated with poor metabolic control.

Treatment with ACE inhibitors before pregnancy along with tight metabolic control in women with diabetic nephropathy, resulting in urinary albumin excretion <500 mg/24 h, has been reported to have a prolonged protective effect on maternal renal function and results in a favorable maternal-fetal outcome without development of preeclampsia (20). Unfortunately, we are unable to support this report, because in our study all women with microalbuminuria or diabetic nephropathy treated with ACE inhibitors before pregnancy demonstrated progression of proteinuria during pregnancy.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Thyroid stimulating hormone dosing in menopausal women for the diagnosis of thyroid neoplasms

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Introduction

The incidence of thyroid cancer in women in Ecuador is 20 for 100.000 inhabitants. This incident increases at the ages of 35 and is the third most common disease (1) after breast and cervix cancer. The global incidence goes from 5 in South America to the 8.5 in Western Europe and 24.4 in Polynesia (2).
Throughout women's lifetime many events are followed by hormone level variations since puberty until menopause, during the last, thyroid dysfunction comes along with changes in concentration of estrogen levels (3,4). Clinical signs are weakness, lethargy, amenorrhea and depression (7) which may be confused with hypothyroidism, rendering difficult to perform a differential diagnosis.
The purpose of this study is to justify among menopausal women, an algorithm that can be easily performed in order to achieve an early diagnosis of benign or malignant thyroid pathology.
Therefore, this study proposes after detecting high levels of TSH, to perform a thyroid ultrasound and provided that nodules are found, a fine needle aspiration (FNA) as an initial procedure conductive to accurate clinical and histological diagnosis.

Objective

To improve the diagnosis of thyroid neoplasms using TSH routine test in the evaluation of menopausal women.

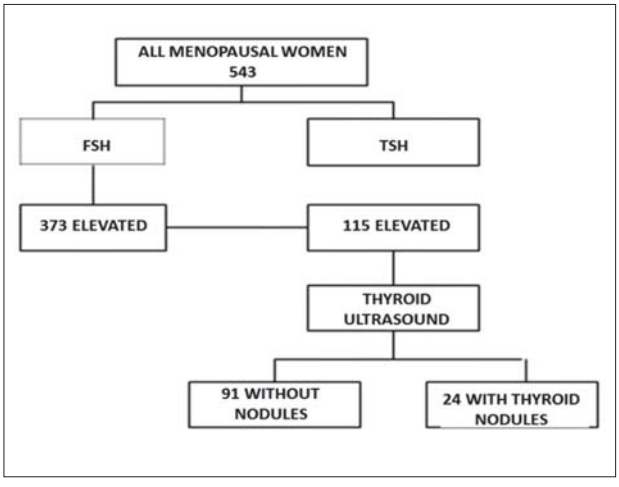
Patients and methods

Descriptive epidemiological study for groups performed at Instituto de Ginecología y Mastología (GINECOMAST) Quito – Ecuador, in which menopausal women according to amenorrhea and FSH levels ≥30 mUI/ml and no clinical signs of thyroid pathology were studied. TSH was measured by RIA (radioimmunoassay), and patients who had a result 4 mUI/ml, were selected for a thyroid ultrasound. Given the presence of thyroid nodules, a FNA was performed and the positive results for malignancy were confirmed by the histopathology after radical thyroidectomy.

Results

Among 543 menopausal patients (amenorrhea) that went through FSH and TSH tests; 373 (68.7%), between 39 and 85 years old (x = 52 years old), were menopausal with elevated FSH, and 115 (30.83%) of them had elevated TSH levels (x=5.64, r=4 -36). The ultrasound found thyroid nodules in 24 (20.86%) pa-

TABLE 1.



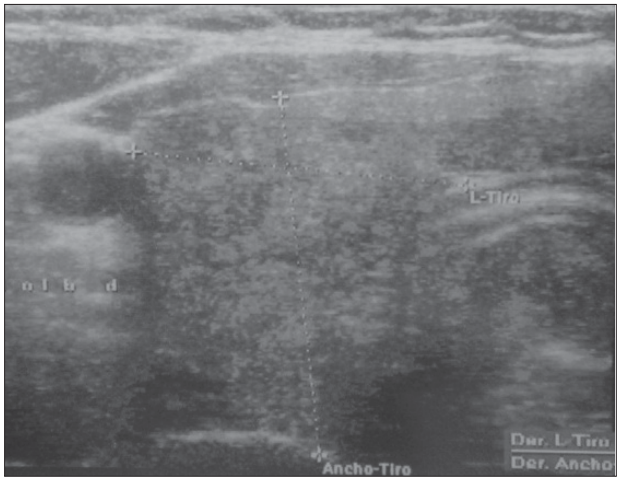


Fig. 1 - Right thyroid lobe of heterogeneous echogenicity, and undefined medial borders, it does not have cleavage plains with the right tracheal wall. Histopathology: Papillary carcinoma.

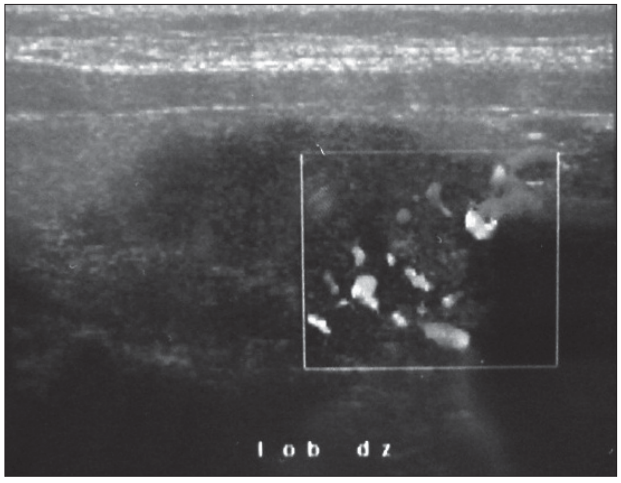


Fig. 2 - Hipoechogenic nodule with very important vascularization on the right thyroid lobe.

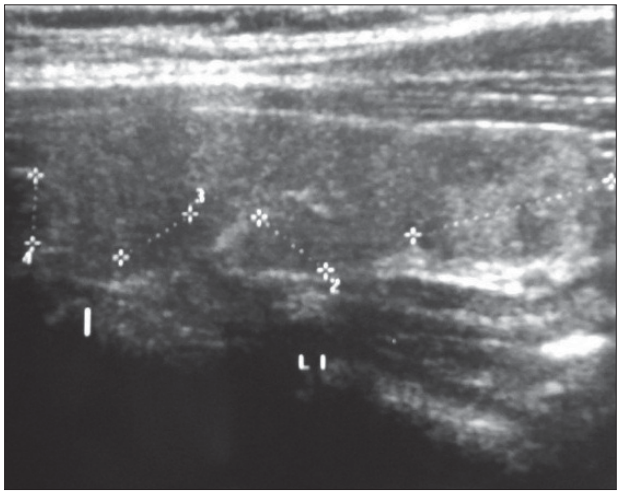


Fig. 3 - Left thyroid lobe with multiple hypoechogenic nodules. HP: nodular goiter.

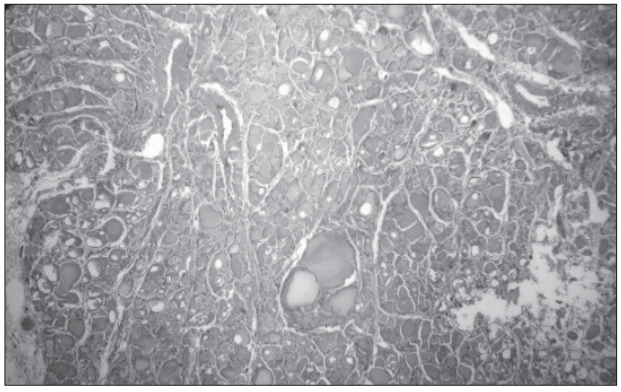


Fig. 4 - Nodular Goiter.- Proliferation of follicles with abundant amount of colloid without cellular atypia.

tients, who had TSH levels much higher than women without nodules (Tab. 1).

The FNA of the nodule was suspicious or conclusive of malignancy in 8 patients (33%), goiter in 14 (58.33%) and follicular adenoma in 2 (8.33%). Five were confirmed as papillary carcinoma (20.83%). Two of them were Hashimoto's thyroiditis (8.33%) and one a benign nodular hyperplasia (4.16%) (Figs 1-6).

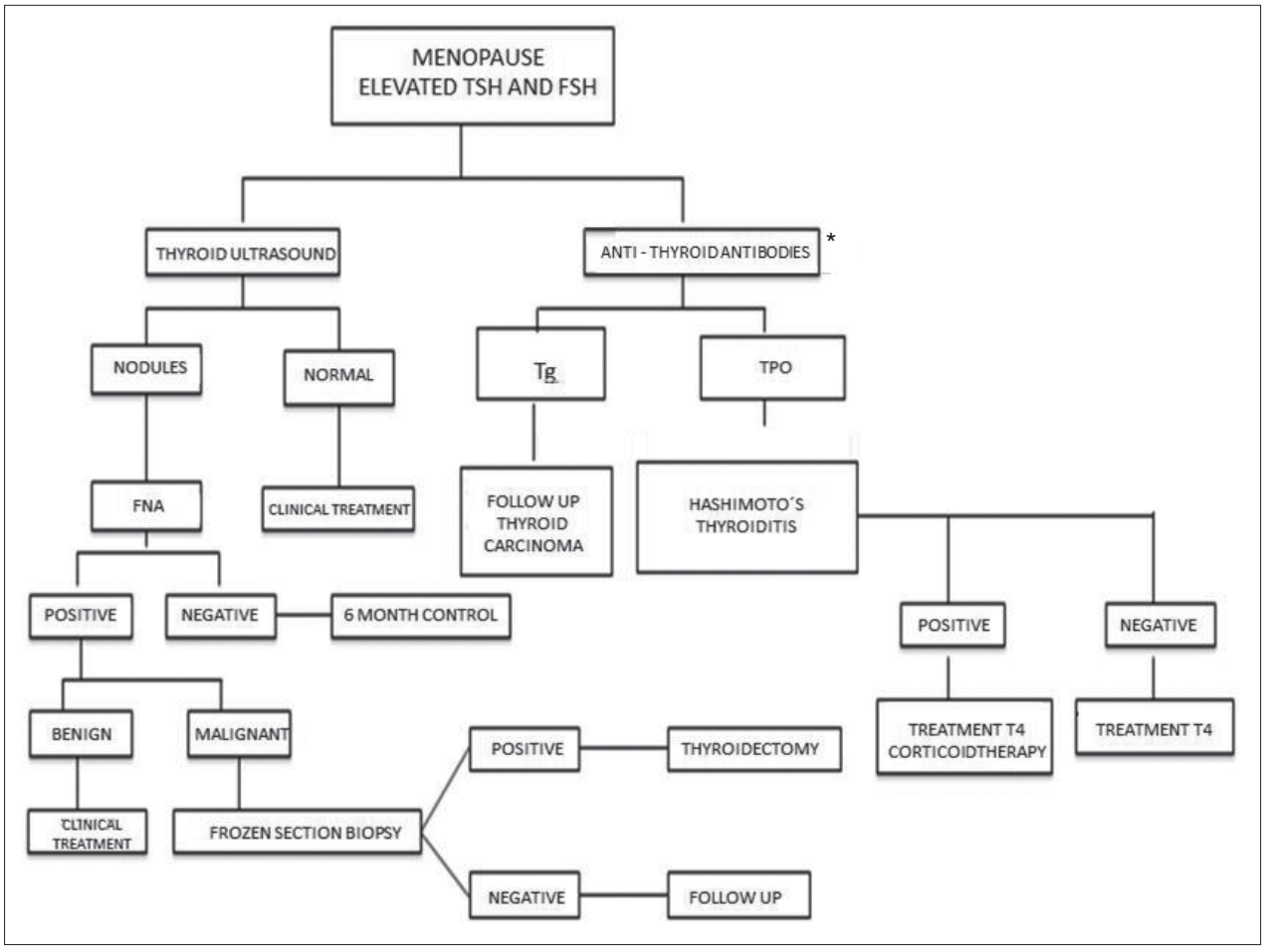
The average of TSH among patients who were diagnosed with cancer was 7.62 (r=6.2-10) significantly higher than patients without thyroid nodules (7.62 vs 5.64; p=0.0034), but similar to the serum TSH values of patients with benign nodules (7.62 vs 8.89; p=0.07).

Discussion

The aging phenomenon is associated with plenty of metabolism disturbances. The hypothalamic and pituitary system cannot avoid these changes and modifications are not exclusive to the reproductive system. Several reports have demonstrated very important changes in the secretion of thyroid stimulating hormone (TSH) throughout our lives (8,9).

The prevalence of hypothyroidism increases as patients grow older and it reaches 10% in older women. The influence of age on the response of the thyroid gland to TSH is not clear. Most elderly people maintain normal T4 plasma levels without a significant change due to age. In this study, we observed an increasing trend in the prevalence of subclinical hypothyroidism in older women, surpassing 40% in women over 55 years. This may be a reflection of the

TABLE 2 – TSH AND FSH ALGORITHM IN MENOPAUSE



Tg: Thyroglobulin
TPO: Thyroid peroxidase (19)
*** The dosage of antithyroid antibodies is still under investigation in our Institute for publication.**

progressive decrease in the secretion of T4 in relation to age (10).

The prevalence of thyroid nodules in the general population depends on the diagnostic method used. Nodules on physical examination are found in 4 to 7% of the population with predominance in females (11), counting on thyroid imaging the prevalence reaches 19 to 67% (12).

It is estimated that 5% of thyroid nodules are malignant (11) and autopsy studies in patients with no history of thyroid disease prevalence is around 49% (13). The vast majority of thyroid nodules are asymptomatic. It is estimated that only 1% of them causes hyperthyroidism (14).

The incidence of thyroid disease in our country justifies the implementation of routine screening for thyroid tumors in menopausal women. Our data suggest that TSH alone should be enough screening provided that the reference values are reliable. World's medical literature considers that 10% of the population might have a thyroid nodule (15), and even if the general population undergoes a thyroid ultrasound, a nodule will be found in about 20 to 45% of women (16) and through autopsy in up to 50% of all cases (16).

The Swedish Sorderstrom, Lowhagen and colleagues in 1952 at th Karolinska Hospital in Stockholm, used the FNA technique for thyroid nodule which was later accepted worldwide (17).

Conclusion

TSH in the routine evaluation of menopausal patients (Tab. 2) allows to select benign or malignant tumor pathology, even if there is no clinical criteria.

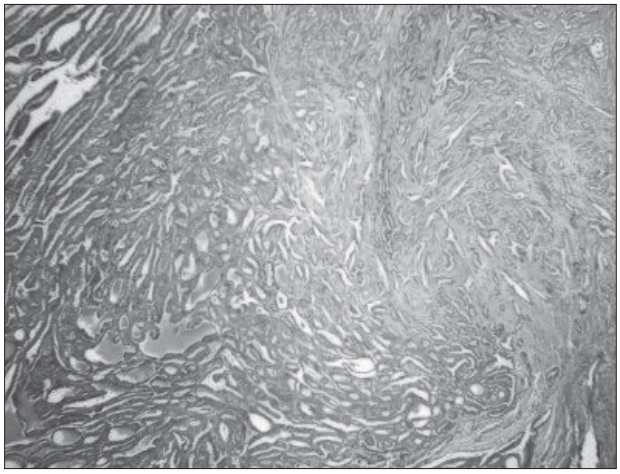


Fig. 5 - Hashimoto's thyroiditis.- Follicles with oncocytic and inflammatory changes of follicular pattern lymphocytic infiltrates.

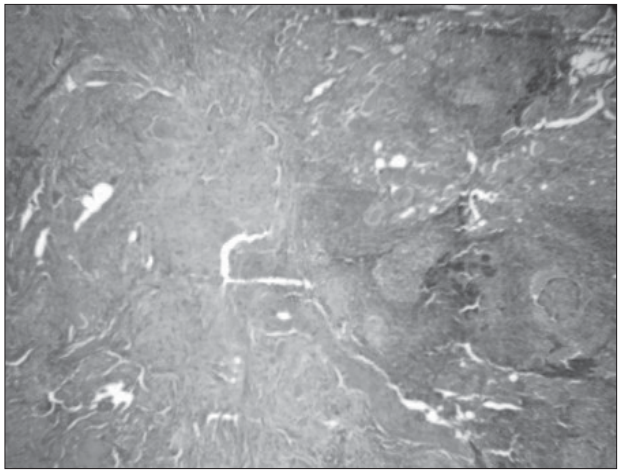


Fig. 6 - Papillary carcinoma.- Papillary pattern proliferation of anaplastic thyroid cells with pseudo-inclusions and "coffee beans like cleft" surrounded by lymphoid follicles

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

The frequency of polycystic ovaries in adolescents. Our experience

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Introduction

The Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder of women in the reproductive age, with a mean incidence of 5-10% (1). The criteria for polycystic ovaries (PCO) as defined by the 2003 Rotterdam consensus are based on the follicle number and ovarian volume, which decrease with age. (2) The syndrome is diagnosed when two of the following three criteria are present (1): Anovulation or oligoovulation (2), biochemical hyperandrogenemia or hyperandrogenism (3), polycystic ovaries observed ultrasonographically (2) PCOS is also accompanied by a number of metabolic disorders, such as insulin resistance and hyperinsulinemia, dyslipidemia and obesity. However, the metabolic manifestations of the syndrome are not included in its criteria.The frequency of the metabolic syndrome in PCOS patients reaches 25% (4). Blood hormone measuements and ultrasound examination of the ovaries are useful in confirming clinical suspicion of the disease. Hyperandrogenism, a high human luteinizing hormone (LH) concentration level compared with human follicle stimulating hormone (FSH), hirsutism are frequent reasons for consultation of teenagers in adolescence and paediatric centers The aim of this study was to elucidate the frequency of the PCO syndrome especially in teenagers without health-problems.

Patients and methods

The study -teenagers were selected from the total population of 140 teenagers, who had completed a menstrual history questionnaire and received no oral contraceptives at the time of the study.
The study-teenagers suffered from abdominal pain depending on various reasons . A group consisting of 56 participants was selecteced from the original group of 140 according to the following criteria oligomenorrhoea, anovulatory cycle using a basal body temperature chart, hirsutism using the clinical Ferriman and Gallway score, increased LH and LH/ FSH ratio >2.5. The sample comprises of 40 healthy teenagers, aged 16-18 years, during a spontaneous menstrual cycle in the department of Adolescence Medicine in Democritus University of Thrace and in others 16 adolescents, aged 15-18 from the Department of Obstetrics and Gynecology in Clinicum Aschaffenburg, Germany during the time from September 2007 until December 2010. The study respondents given were agree to participate in the follow up study for the next five months after the first doctor visiting. The menstrual cycle patterns were defined as follows: *regular menstrual cycles*: an average cycle length between 22 and 41 days; no more than a single cycle with a length of less than 22 or greater than 41 days during the past year; *irregular menstrual cycles*: an average cycle length between 22 and 41 days; two or more cycles with a length of, 22 or .41 days during the past year; *oligomenorrhea*: an average cycle length between 42 and 180 days;Transvaginal sonography was performed between cycle day 7 and 10 to determine the follicle number and revealed more than 10 follicles of 2-8 mm in diameter, typically distributed peripherally and central stroma was increased (3,4). Hormone levels (FSH, LH, DHEAS) were measured on day 6 or 7 after menses.

Results

The teenager-participants were monitored for about 5 months. The clinical characteristics of the teenagers

are shown in table 1. Most PCO subjects had anovulatory cycles, hirsutism and oligomenorrhea.

TABLE 1 - CLINICAL LEADING SYMPTOMS OF PCOS.

Anovulation	66.6%
Hirsutism	30.5%
Acne	29.4%
Obesity	20.6%
Irregular cycles	82.5%
Oligomenorrhea	90.5%

LH and androgen concentrations were significantly higher in teenagers with PCO. Oligomenorrheic participants with PCO had the highest LH levels.

TABLE 2 - LABORATORY FINDINGS OF PCOS.

Hormones		
LH	> 12 IU/I in (40/56) cases	71.4%
	> 16 IU/I in (16/56) cases	28.6%
LH/FSH ratio	> 2.5 in (50 /56) cases	89.2%
DHEAS	> 8.9 µmol/ l in (50/56) cases	89.2%

Elevated LH levels were found to represent the most common endocrine abnormality in oligomenorrheic adolescents with an incidence 42%. In our study high LH levels were associated with high androgen levels and the prevalence of PCO in our population was 42%.

Discussion

The present study demonstrates the prevalence of PCO in a general population of adolescence. PCO was found to be very common in young women. The pathogenesis of PCOS is possibly based on interactions between genetic and certain environmental factors. Although our sample of adolescents is small, our findings suggest clearly relationship between PCO syndrome and menstrual cycle pattern as well as elevated LH concentration. In our collective we found high androgen levels but the role of hirsutism and acne is unclear. Consonant to our findings was the PCO prevalence found in other studies in teenagers and adults with oligomenorrhea. (45%) (6). The PCO prevalence in adults with oligomenorrheic populations is higher referred according to same authors, although are used the same PCO definitionscriteria (7-9) PCO is associated with irregular menstrual cycles, oligomenorrhea, high LH and androgen levels but their relationship in adolescents has been not satisfactory documented (10). The recognition of the early signs of PCOS during or even before adolescence is of great importance. In young women with hyperandrogenism it is important to establish the correct diagnosis for PCOS and rule out other causes of androgen excess. Early treatment is crucial to prevent the long term complications of the syndrome, especially infertility and cardiovascular disease. Treatment of PCOS in adolescent girls should aim in achieving ovulation, normalizing the menstrual cycle, reducing and if possible eliminating hirsutism and acne, losing weight and treating hyperlipidemia and hyperglycemia in order to reduce the risk of cardiovascular disease. Further research is necessary to elucidate how PCOS behaves and disturbs the endocrine harmony of the young female body in that transitional place of a woman's life.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Efficiency of combined therapy for treating symptoms of endometriosis

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Introduction

Combined therapy (surgery with consequent glandular therapy) is, in the opinion of majority of researchers, most effective in treating endometriosis (Krasopolskiy et al., 2000, Adamyan L.V., 2005). However, many doctors and patients in Kazakhstan and Middle Asia prefer only surgery with laparoscopic access. In this connection, substantiation of combined therapy is very important as mandatory for treating symptoms of endometriosis.

Aim

Definition of efficiency of combined therapy for reducing symptoms of endometriosis.

Patients and methods

For the period from March 2008 until March 2011 120 women with histologically confirmed external genital endometriosis with 1,2,3 stages of diffusion in accordance with classification R-AFS (Revised Classification of American Fertility Society, 1985) were clinically examined and treated. 60 patients had only endosurgery treatment (I group). 60 patients received glandular therapy by dufaston - 10 mg 3 times per day from 5 to 25 day of the cycle for 6 month after endosurgery (II group). Surgery was made on the 6-8 days of menstrual cycle with the use of operational block of «Storz» firm (Germany), mono-polar coagulator.
Average age of patients in research group sequaledto (34,6±1,1) years and (31,6±1,4) years accordingly.
Examined and treated patients had the following forms of endometriosis: ovary endometriosis 43.3% (52); peritoneal form of endometriosis 45.0% (54); combined from (ovary endometriosis and peritoneal form) 11.6% (14).
In the 1st group, 1 stage of endometriosis diffusion was observed at 35.0% (21), 2 stage at 35.0% (21) and 3 stage at 30.0% (18) of the patients. In the 2nd group, 1 stage of endometriosis diffusion was diagnosed at 40.0% (24), 2 stage at 30.0% (18) and 3 stage at 30.0% (18) of the patients.
For estimating efficiency of therapy, were estimated the basic clinical symptoms: pain syndrome, and menorrhagia. Expression of pain syndrome was estimated on visual-analogues scale (0 mm – absence of pains, 100mm – acute pain) at the beginning and end of research. The results of research were statistically processed.

Results

Pain syndrome was observed at 90.4% (108) of patients, involved in the research, with this we had high rate of dysmenorrhea (51.9%), as well as continuative pelvis pains (47.5%), dyspareunia was observed at 32.8% of patients. Average intensity of pain syndrome before beginning of treatment equaled to 70mm on visual analogue scale in both of group.
Before treatment in the 1st group patients the pain syndrome was observed at 91.7% (55), here with at 90.5% (19) with 1 stage, at 90.5% (19) with 2 stage and at 94.4% (17) patients with 3 stage of the disease. Intensity of pain syndrome on visual analogue scale equaled to 55mm, 70mm and 80mm accordingly on

stages of endometriosis diffusion. Pains were preserved at only 61.9% (13) patients with 1 stage, at 81.5% (17) patients with 2 stage and all of the patients with 3 stage of disease - 94.4% (17). With 1 stage of endometriosis diffusion intensity of pain syndrome was reduced to 25mm, at the 2 stage of endometriosis diffusion to 35mm, at the 3 stage it was reduced to 47mm. The average intensity of pain was 35 mm after treatment instead of 70 mm before the treatment. Thus, the effect of treatment were at 28.6% (6) of patients with 1 stage, at 9.5% (2) patients with 2 stage. Among the patients with 3 stage of endometriosis didn't have the effect of treatment. Therefore, in the 1st group only 14.5% (8 out of 55) of patients had effect (absence of pain) of surgery therapy. Despite the small percentage of effectiveness, pain intensity decreased an average of 2 times.

In the 2nd group patients the pain syndrome before treatment was observed at 88.3% (53), herewith at 83.3% (20) with 1 stage, at 88.9% (16) with 2 stage and at 94.4% (17) patients with 3 stage of the disease. Intensity of pain syndrome on visual analogue scale equaled to 55mm, 75mm and 80mm accordingly on stages of endometriosis diffusion.6 months after treatment, this syndrome was observed at 33.9% (18 out of 53) of patients. During the treatment, ranging from 1 month, pain disappeared in most patients with stage 1 and the end of treatment was observed in 12.5% (3 patients). In patients with second and third stages of endometriosis pain syndrome was observed in 33.3% (6) and 52.9% (9), respectively. Pain intensity decreased to 10 mm - 1 Stage, 15mm - 2 stage, 25mm - 3 stage of endometriosis diffusion. The average intensity of pain was 17mm after treatment, instead of 70 mm before treatment. Thus, the effect of treatment for1 stage was obtained in 70.8% (17), at 2stage in 55.6% (10), at 3 stage in 44.4% (8). So at 66.0% (35 out of 53) of patients 2st groups the effect of the therapy was observed and pain intensity decreased by 4 times.

Menorrhagia were observed at 34.2% (41) of studied patients. In the 1st group before treatment menorragiawere at 35.0% (21) patients: at 1 stage – 23.8% (5); at 2 stage – 33.3% (7), at 3 stage– 50.0% (9). After treatment in that group menorrhagia were preserved at 21.7% (13) of patients: at 1 stage – 9.5% (2); at 2 stage – 14.3% (3), at 3 stage – 44.4% (8). In the 2nd group before treatment this symptom were at 33.3% (20) patients and 15.0% (9) patients after therapy. At 1 stage menorrhagia were at 16.7% (4) instead of 4.8% (1) patients after therapy. At 2 and 3 stage were 33.3% (6) and 55.6% (10) of patients who had these symptoms before treatment. After 6 months it was at 16.7% (3) and 27.8% (5) of patients with 2 and 3 stage, respectively.

Analysing the data, we can say that the significant decrease of the main symptoms of the disease ($p<0,01$) was noted in all types of therapy. However, the combined method of therapy turned out to be most effective in reducing symptoms of disease. In it, clinical symptoms after treatment were less frequent, than the ones after surgery ($p=0,01$). Clinical efficiency of combined treatment was for the most part related with reduction of pain syndrome. Was noted direct correlation dependence between endometriosis stage and results of therapy. The heavier pathologic process is, the less therapy is efficient ($r=0,98$).

Conclusions

Comparative estimation of efficiency of methods of endometriosis treatment allowed to state, that combined therapy turned out to be more efficient, than endosurgery at all stages of pathologic process. Results of treatment of genital endometriosis are directly proportional to its stage: the more diffusive endometriosis is, the less therapy is efficient. Using dufaston after surgery reduces frequency of endometriosis symptoms and improves life quality of women.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
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Efficiency of surgery and glandular therapy of endometriosis

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Introduction

Endometrioma (ovary endometriosis) is the most frequent form of endometriosis at women in reproductive age. Treatment of endometriosis cysts is in removing them by surgery with laparoscopic access. However, relapses of disease are observed after surgery (1,2). Different methods of removing cysts are used during surgery. Accessible literature lacks information about efficiency of different methods of removing endometriosis cysts. For preventing relapse, glandular therapy is offered (3,4). But many doctors and patients prefer only surgery method of treating endometriosis.

Purpose of research

Providing basis for efficiency of after-operational glandular therapy for reducing pain syndrome and relapse of endometrioms (ovary endometriosis).

Subject and methods of research

Since November 2005 until May 2009 we had laparoscopic removal of symptomatic endometriomas at 95 patients 24-35 years old. Diagnosis is histologically verified. During surgery were held ovary resection 40, cyst enucleation 20, adnexectomy 17, endocoagulation for 18 patients. Accordingly, in 33 cases was made adhesiolysis. Surgery was made with use of coagulator of mono-polar type of high-frequency endosurgery on operational laparoscopic block of «Storz» firm (Germany). After surgery 33 patients received low-dose combined oral contraceptive (COC), containing desogestrel, in contraceptive mode, for 6 months. 30 patients received didrogesteron (dufaston) in dose 10 mg 3 times per day from 5th to 25th day of the cycle for 6 months. The rest 32 patients refused glandular therapy. Thus, all patients, depending on method of therapy, were divided in 3 groups: 1st group - 32 patients, not receiving anti-relapse glandular therapy, 2nd group-33 patients, who received COC after endosurgery, 3rd group - 30 patients, who received didrogesteron. Efficiency of therapy was estimated on reduction of frequency of main syndromes of disease (pains and dismenorrhea), on stage of reduction of pain according to Visual Analog Scale and relapse frequency in 12 months after therapy. Intensity of pain syndrome was estimated on VAS (0mm – absence of pain, 100mm – acute pains) at the beginning and end of therapy. Results of research were undergoing statistic processing.

Results of research

Age of patients, involved in research, varied from 19 to 35 years. Pain syndrome was observed at all the patients before surgery. Intensity of the pain syndrome was estimated at 88.6 mm average. Irregularity of menstrual cycle was observed at 45 patients, equaling to 47.4%.

In the 1st group of women, preservation of pain syndrome after endosurgery was observed at 34.4% (11) of patients, in 12.5% (before surgery observed at 11 out of 32) –irregularity of menstrual function. Pain intensity was estimated at 46 mm average instead of 91 mm before therapy.

Depending on volume of surgery, patients of the 1st

group showed the following results. Pain syndrome was preserved at 50% (7 out of 14) patients after ovary resection, at 28.6% (2 out of 7) after adne sectomy, at 12.5% (1 out of 8) after cyst enucleation and at 25.0% (1 out of 4) of patients after endocoagulation. Relapse of endometrioma after 12 months was observed at 18.2% (6). Relapse of disease was observed at 21.4% (3 out of 14) of patients after ovary resection, at 14.3% (1 out of 7) of patients after adnexectomy, at 12.5% (1 out of 8) after cyst enucleation, at 25.0% (1 out of 4) after coagulation of endometrioid heterotopia.

In the 2nd group of women preservation of pain syndrome was observed at 18.8% (6), menstrual irregularity at 9.3% (before surgery observed at 17out of 32). Intensity of pains was estimated average at 32 mm (before surgery it was equal to 88 mm). Relapse of disease was observed at 12.5% (4) cases. Relapse of disease was observed at 2 out of 11 (18.1%) patients, who had ovary resection, at 1 out of 5 after adnexectomy, at 1 out of 9 after coagulation of endometroid heterotopias and in no case out of 5 after cyst enucleation.

In the 3rd group of women after 12 months of treatment, pain syndrome was preserved at 16.7% (5)patients, at 10% (before surgery was observed at 17 out of 30) – irregularity of menstrual cycle. Intensity of pain syndrome was estimated at 39 mm (before surgery it was estimated at 87 mm). Relapse of disease was observed at 13.3% (4) cases. Relapse of disease was observed at patients, who had ovary resection 2 out of 15 (13.3%), after adnexectomy at 1 out of 5, aftercoagulation at 1 out of 5, at none of 5 after cyst enucleation.

Results received state, that after endosurgery (first group) pain syndrome was preserved at every third patient (34.4%) and was observed 15.6% more frequent, than at patients, who received COC after operation and 17.7% more frequent, that at patients, who received gestagenafter operation. Regarding intensity of the pain syndrome, it was reduced by the end of 12 month in all groups. However, at the patients with endosurgery it was reduced at 1,3 time, at combined therapy with COC by 2,6 times, at combined therapy with gestagen by 2,2 times.

Relapses of disease were more frequently observed at patients after surgery – at 18.2% of patients versus 12.5% at usage of COC after surgeryand 13.3% at usage ofgestagen after surgery. For the most part, relapses are observed at patients, who had ovary resection (at 10 out of 40 patients, who had resection, i.e. 25%), with that, without usage of anti-relapse glandular therapy (21.4% versus 18.2% and 13.3% in 2nd and 3rd groups). As we can see from this data, frequency of relapse of ovary endometriosis, was lesser after ovary resection at patients, who received gestagen.

Conclusion

Pain syndrome after one year was more frequently preserved at patients with endometriosis, who had only surgical removal of ovary endometriosis (p=0,01). Efficiency of surgery with consequent receipt of hormones turned out to be better, independent of type of glandular therapy in the sphere of reducing frequency of pain syndrome. Intensity of pain syndrome was reduced at all types of therapy. However, after endosurgery receiving COC and gestogen allowed reduce frequency of pain syndrome more, than at using only surgery.

Thus, using glandular therapy after surgical removal of ovary endometriosis allows reduce frequency, intensity-of pain syndrome and frequency of relapse of disease.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Metabolic effects of the levonorgestrel releasing-intrauterine system on long term users in Latvia

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Introduction

Cardiovascular disease (CVD) claims more female lives than cancer and accidents combined in Latvia. In 2006 CVD caused death for 73% of Latvian women (58% men). CVD related death-rate is higher and the average life expectancy of patients is lower than in most of the European countries (1).
With increase of diabetes, obesity and metabolic syndrome CVD will continue to rise. The share of obese persons in the age group of 25-44 year-olds is 9.6%, and in the age group of 45-64 year-olds - 31% (2). For women there is a clear pattern: share of obese persons increases with age. The levonorgestrel-releasing intrauterine system (LNG IUS) has been approved for contraception and heavy menstrual bleeding therapy in Latvia since 1998. The LNG IUS use is increasing, particularly in the treatment of endometriosis, endometrial hyperplasia and even endometrial cancer (3). Since 2006 LNG IUS has been included in the list of reimbursable drugs in Latvia with the indication N92 - excessive, frequent and irregular menstruation (ICD-10). Each year about 1500 LNG IUS are administered to patients having this diagnosis. Contrary to other progestogen-only contraceptives the activity of LNG IUS is based on a local effect on the endometrium and non-essential systemic effects. The effects on various metabolic indicators have been studied for young women using LNG IUS for contraception (4) and for women suffering from heavy menstrual bleeding (5). Poor patient compliance to this treatment is common as patients are afraid of systemic effects caused by hormonal preparations, mainly the weight gain.
The purpose of the study was to clarify the metabolic characteristics of long-term LNG IUS users and the possible LNG IUS impact on these parameters.

Materials and methods

102 women, who had LNG IUS inserted at I.Vasaraudze's Private Clinic Ltd during 01.01.1998 - 31.01.2011 were invited to participate in a retrospective descriptive study. The examination plan included: determining the BMI, the measurement of arterial blood pressure and abdominal circumference, as well as the collecting of blood count, where the fasting glucose level, C peptide level, total cholesterol (TC), high density cholesterol (HDC), low density cholesterol (LDC), triglyceride levels, thyreotropin level, follicle stimulating and luteinizing hormone level were determined. The HOMA Calculator© The University of Oxford 2004 was used in order to determine the beta cell function and insulin sensitivity. The exclusion criteria were as follows: LNG IUS was not used any more or it was not possible to carry out the planned examinations for some reason. The patients were dividend in groups depending on their BMI. Women received electronic invitation letter and no payments or gifts were offered for participation. The patients paid for all the examinations. Prior to the study an informed patient consent was obtained to use the clinical information gathered for statistical analysis and publication. This study was approved by the Ethics Committee of Riga Stradins University.

Data analysis
Microsoft Excel was used in creating the database and producing graphs while the data were analyzed using

Statistical Package for The Social Sciences (SPSS) version 17 for Windows. The commonly used p<0.05 rule was applied to detect significant differences.

Results

102 invitations were sent; 54 women (52,9%) agreed to participate in the study; 15 were excluded from the study since they did not want or could not do the blood tests. 39 women (31%) underwent all the examinations, and the analyses were based on this sample. The patients were randomized into three groups: Group 1 (BMI 18,5-24.90) - 18 women (49%); Group 2 (BMI 25-29,9) - 19 women (49%); Group 3 (BMI over 30)- 2 women (5%).
22 women (56%) were using LNG IUS for contraception, 17 women (46%) – for heavy menstrual bleeding therapy. The average age of the women was 42,7 (SD =7,31 years), without statistically significant difference between the groups (p=0.141), the oldest users were in the BMI Group 3. The total indicators referring to demographic characteristics and biochemical analyses are given in tables 1 and 2.
Table 1 data does not show any statistically significant differences between the groups based on the indicators of age (p=0.1941), LNG IUS use duration (p=0.8710), systolic blood pressure (p=0.0607) and diastolic blood pressure (p=0.0215). The highest indicators were found in BMI Group 3.
As Table 2 shows, BMI Group 1 had a statistically significant lower level of LDL (p=0.0007); cholesterol (p=0.0040), triglycerides (0.0425), HDL (p=0.5937), glucose (p=0.9659), C peptide (p=0.8273), HOMA-IR (p=0.9594), TSH (p=0.6237), FSH (p=0.3751) and LH level (p=0.3206) did not have a statistically significant difference between the groups, while the highest indicators were in BMI group 3.
By means of the method of linear regression it was identified that the increase in BMI leads to a statistically significant increase in glucose level (p>0.003) and insulin resistance indicators (>0.001); the increase in glucose level results in the increase of insulin resistance (p>0.001).

Discussion

According to the results of the present study, LNG IUS does not have a systemic impact on metabolic pa-

TABLE 1 - BASELINE CHARACTERISTICS OF THE STUDY GROUP.

	Total group	Group1	Group2	Group3
Age (years)	42.7±7.31	41±1.54	44.15±1.80	45.5±7.32
Height (cm)	171(164-183)	172(164-182)	169(164-184)	169(164-174)
Weight (kg)	73.03((53.6-93.4)	67.1(53.6-76.6)	76.8(66-92.8)	93.03(92.7-93.4)
LNG IUS use duration (months)	53.02(6-150)	50.61(6-150)	48.36(6-144)	84(54-114)
Systolic BP (mmHg)	119.46(100-180)	114.27(100-130)	122.21(100-150)	140(100-180)
Diastolic BP (mmHg)	77.53(58-110)	72.11(58-80)	81.36(60-110)	90(70-110)
Abdominal circumference (cm)	86.47(76-95)	83.88(76-94)	88.31(79-95)	93(92-94)

TABLE 2 - THE METABOLIC INDICATORS OF THE STUDY GROUP.

Variables	Mean±Standard deviation (SD)			
	Total group	Group1	Group2	Group3
Cholesterol (mmol/L)	5.2±0.96	4.74±0.91	5.69±0.80	5.48±1.1
Triglycerides (mmol/L)	1.02±0.58	0.78±0.37	1.2±0.67	1.35±0.81
HDL (mmol/L)	1.65±0.36	1.70±0.38	1.62±0.37	1.46±0.16
LDL (mmol/L)*	3.05±0.90	2.53±0.78	3.54±0.71	3.31±0.10
Glucose (mmol/L)	5.15±0.67	5.06±0.45	5.05±0.69	6.62±0.10
C peptid (ng/mL)	1.66±0.67	1.54±0.50	1.59±0.66	3.01±0.57
HOMA-IR	1.26±0.48	1.169±0.29	1.176±0.45	2.4±0.69
TSH (mU/L)	1.54±1.17	1.68±1.26	1.47±1.17	1.11±0.26
FSH (U/L)	15.28±20.59	11.06±15.58	17.25±22.98	32.25±37.40
LH (U/L)	8.20±8.41	6±5.68	8.81±9.27	19.9±13.01

* Significantly different at the 5% level of significance.

rameters. Changes in the metabolic parameters do not depend on the duration of LNG IUS use.

54% of the women in the sample had an increased BMI reflecting the statistical situation in the country. There is a direct correlation between an increase in the glucose level and insulin resistance, along with the changes in BMI.

An increased BMI was observed among older patients, also observing an elevated level of gonadotropin. The LNG IUS are administered primarily to women over 40, the same age group where the most significant changes in metabolic characteristics have also been indentified. The design of the present study did not allow us to evaluate whether changes in the gonadotropin level are connected with the use of LNG IUS. Since the number of LNG IUS users undergoing heavy menstrual bleeding therapy is increasing every year, additional evaluation of this correlation is necessary.

This study shows significant relationships between BMI, glucose level and insulin resistance. The measurements of the BMI and abdominal circumference could be included in the annual examination plan of LNG IUS users in order to select a group of patients for further, more extensive examination in a timely manner.

Conflict of interest

All authors declare that they have no conflict of interest.

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Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

Natural progesterone treatment in preterm labor

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Background

Preterm birth 22+0 – 36+6 gestational weeks occurs in 5% in developed countries and up to 25% in developing areas and is the cause of 70% of all perinatal mortality and morbidity. Risk factors for spontaneous preterm birth include poor nutrition, low socioeconomic status, smoking, prior preterm birth, subplacentar bleeding, systemic or urogenital tract infection or inflammation, cervical or uterine anomalies and psychophysical maternal stress.
Synthetic progestins and natural progesterone have been widely used as luteal phase support in patients with recurrent miscarriages and in assisted reproduction since the 1950s, and for preventing premature birth since the 1960s. Prophylactic weekly intramuscular injections of 17α-P significantly prevented the incidence of premature birth and to improve early neonatal outcome in comparison to placebo in patients with a medical history of a previous premature birth, and in asymptomatic patients with a sonographically short cervix. Prophylactic daily doses of a vaginal gel or tablet containing natural progesterone significantly reduced the incidence of premature birth and improved early neonatal outcome as compared to placebo in patients with a previous preterm birth, prophylactic cervical cerclage or uterine malformation and in asymptomatic women with a sonographically short cervix, but was not proven to be efficient in the largest randomized controlled trial.
Only a few studies have investigated the effect of progesterone treatment in women who remained undelivered after successful parenteral tocolysis because of preterm labor. In one study 60 women with preterm labour (24-34 gestational weeks) were randomized to receive either an intramuscular injection of 341 mg of 17α-P twice a week or observation alone after atosiban infusion. In another study 70 women with preterm labor (24-34 gestational weeks) were randomized to either receive a progesterone suppository 400 mg daily or to observation after magnesium sulphate infusion.
Human studies have focused on early neonatal outcome, i.e. mortality, morbidity and neonatal intensive care unit (NICU) admittance within the first week of life. Further studies are required concerning natural progesterone, and to establish the optimal dose and type of agent as well as long term effects on the newborn. In our study, daily progesterone vaginal gel 90 mg after successful parenteral tocolysis in 60 women with a singleton pregnancy, premature uterine contractions between 24 and 34 gestational weeks and a cervical length <25 mm will be presented. Clinical maternal and neonatal outcome, infant well-being at 6 and 12 months of age, plasma levels of steroid hormones, pro-inflammatory and anti-inflammatory cytokines are monitored.

Comment

Data from animal studies suggest, that natural progesterone is more efficient than 17α-P in preventing premature ripening of the cervix uteri. Progesterone, like androgens and corticosteroids, exert anti-inflammatory effects and is a strong immunosuppressor. Preliminary data suggests that progesterone, which in contrast to 17α-P has no androgenic effects, also has fewer side effects than 17α-P. The genomic and non-genomic effects of progesterone and possible adverse effects of tocolysis and progesterone treatment will be discussed.

Giorn. It. Ost. Gin. - Vol. XXXIV - n. 1
Gennaio-Febbraio 2012

How does the thyroid function influence the effectiveness of infertility treatment with ART?

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Introduction

In the last decades we can see a rapid increase of various thyroid disease rates in Ukraine. Infertility rates are stabile high. This leads to the increase of quantity of women with thyroid diseases who want to become pregnant. Sometimes the only way to achieve pregnancy is ART treatment.
There are various definitions for ART. According to the definition used by CDC, ART includes all fertility treatments in which both eggs and sperm are handled; ART procedures involve surgically removing eggs from a woman's ovaries, combining them with sperm in the laboratory, and returning them to the woman's body or donating them to another woman; they do not include treatments in which only sperm are handled (i.e., intrauterine–or artificial–insemination) or procedures in which a woman takes medicine only to stimulate egg production without the intention of having eggs retrieved. In Ukraine, nevertheless, all the above mentioned methods of treatment are meant as ART. In this study we use "ART" only for the IVF treatment.
It is also discussed what method is the best for calculating fertility success rates. In our study we use the pregnancy rate per one stimulation cycle as the criterion for fertility success rates.
The aim of our study was to examine the influence of the thyroid function on the effectiveness of infertility treatment with ART, that is IVF.

Materials and methods

A retrospective analysis of 130 cases of infertility treatment with ART in the department of diagnosis and treatment of infertility (Donetsk regional center of motherhood and childhood protection, Donetsk, Ukraine) during 5 years (2006-2009). The patients were divided into 2 groups:

1) 65 women - with thyroid pathology;
2) 65 women - with normal function of the thyroid gland.

In both groups we analyzed the pregnancy rates following ART and also the frequency of different diseases of the thyroid gland in the first group.

Results

We found that the thyroid pathology had influenced the effectiveness of infertility treatment with ART. The pregnancy rates were almost 20% lower in the group of infertile women with thyroid pathology compared with the group of infertile women with normal thyroid function, $p \geq 0,05$.
When women with thyroid dysfunction started their infertility treatment, they had different thyroid diseases in different combinations (goiter, chronic thyroiditis etc), but their thyroid function was as follows: euthyroid – almost 75%, subclinical hypothyroidism – almost 15%, hypothyroidism – almost 10% of women. ART treatment could only be started when hypothyroidism wascompensated. So we can see that even euthyroid status influenced the effectiveness of the ART treatment in the group of women with thyroid diseases.

Conclusions

The thyroid function influences greatly the effectiveness of infertility treatment with ART. We suggest an obligatory screening (TSH and aTPO) of thyroid function among infertile women in order to find and treat the thyroid pathology before the treatment with ART. It is also necessary to find new methods for increasing the ART success rates in women with thyroid dysfunction, because even euthyroid status by chronic thyroiditisdecreases pregnancy rates significantly.

AUTHOR'S INDEX

Notes

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