TESIS DOCTORAL
Antagonistas de la GnRH en los tratamientos de reproducción asistida.

José Luis Gómez Palomares

Directores:
Eleuterio R. Hernández de Miguel y
José Schneider Fontán

Departamento de Ciencias Médicas y Quirúrgicas
Universidad de Cantabria

FivMadrid, Centro de Reproducción Asistida

•Santander, 2012•

joseluisgomezpalomares@yahoo.com

A Pedro Gómez Gallego, mi padre.

Índice de contenidos

Índice de abreviaturas

E2.....Estradiol.

FIV.....Fecundación in vitro.

FSH.....“Follicle-stimulating hormone”, hormona folículo estimulante.

FSHr.....FSH recombinante.

GnRH.....“Gonadotropin-releasing hormone”, hormona liberadora de gonadotropinas.

hCG.....“Human chorionic hormone”, hormona coriónica humana.

HMG.....“Human menopausal gonadotropin”, gonadotropina humana de la menopausia.

IA.....Inseminación artificial intrauterina.

LH.....“Luetinizing hormone”, hormona luteinizante.

NS..... No significativo.

SHO.....Síndrome de hiperestimulación ovárica.

SOP.....Síndrome de ovarios poliquísticos.

UI.....Unidades internacionales.

Introducción

Los inicios.

La disfunción reproductiva es la incapacidad de una persona para reproducirse (Hernández, 2011). Procrear, engendrar nuevos individuos de una misma especie, es una necesidad para la mayoría de los seres humanos. Incluso para algunos grupos religiosos como los cristianos adquiere el carácter de imperativo divino ("Creced y multiplicaos", Génesis 1:28).

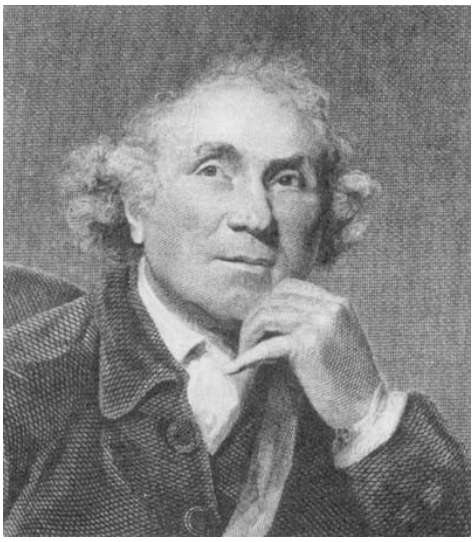


Figura 1: John Hunter (1728 – 1793). Realizó la primera inseminación artificial con éxito.

Aunque ya se habían hecho intentos anteriores en perros, fue John Hunter (Figura 1) quien por primera vez, en 1785, llevó a acabo con éxito una inseminación artificial humana (IA). Se trataba de una pareja en la que el varón había nacido con hipospadias y era incapaz de completar una relación sexual. Hunter realizó el procedimiento utilizando una pluma de ave (O'Dowd y Philipp, 2000).

Tuvieron que pasar casi dos siglos para que se produjeran, en las últimas décadas, los avances y descubrimientos que, de forma conjunta, permitieron el desarrollo de diversos tratamientos para la ayuda a la reproducción humana. Uno de estos avances fue el desarrollo, por Rosalyn Yalow y su equipo (Figura 2), de las técnicas para medir las hormonas mediante radioinmunoanálisis por lo que recibió el premio Nobel de Medicina en 1977. Previamente, en 1949 se aisló por primera vez de la orina de mujeres menopáusicas la HMG (human menopausal

gonadotropin), que corresponde a la FSH y LH urinarias (Martínez, 2011) y que se comercializó con el nombre comercial de Pergonal® (Figura 3). Bruno Lunenfeld en la década de los 60 del pasado siglo publicó los primeros embarazos tras la inducción de la ovulación con esta gonadotropina (Lunenfeld, 2004).

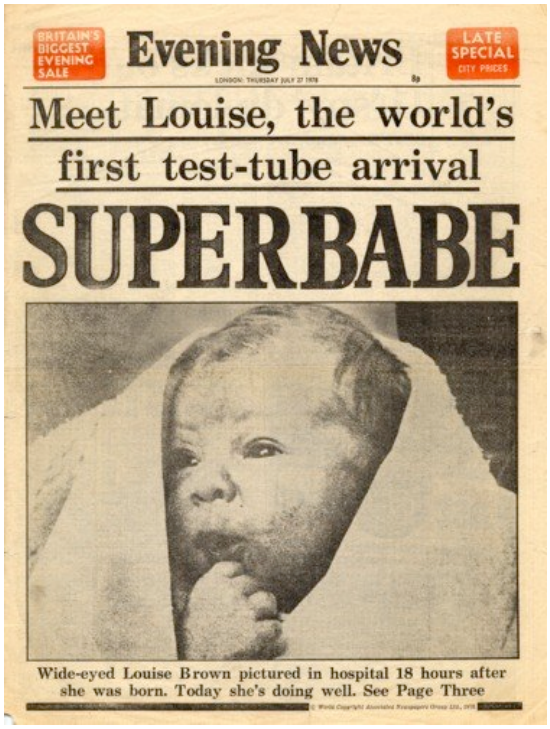
Figura 2: Rosalyn Yalow (1921-2011). Galardonada con el Premio Nobel de Medicina en 1977 por desarrollar las técnicas de radioinmunoananálisis que permitieron cuantificar las hormonas peptídicas.

En 1973, el equipo de Carl Wood, en la Monash University de Melbourne, Australia, logró el primer embarazo obtenido gracias la fecundación extra-corpórea, lo que hoy conocemos como fecundación i*n vitro* (FIV). La gestación no evolucionó adecuadamente y la madre no llegó a dar a luz. Cinco años después, Robert Edwards (embriólogo) y Patrick Steptoe (ginecólogo) en el Reino Unido consiguieron que, el 25 de julio de 1978, naciera Louis Brown (Figura 4). El primer ser humano concebido mediante FIV. Estos investigadores fueron galardonados, en la persona de Robert Edwards con el Premio Nobel de Medicina en 2010.

Figura 3: Pergonal. La primera gonadotropina disponible comercialmente.

El descubrimiento de las técnicas de DNA recombinante permitió que se creara otra vía para la síntesis de gonadotropinas a parte del aislamiento de la orina de mujeres postmenopáusicas. En este caso el gen que codifica la FSH se inserta en un plásmido que por transfección se introduce en una línea celular de ovario de hámster (Lunenfeld, 2004). La primera gonadotropina

recombinante que estuvo disponible en el mercado, en 1995, fue la folitropina alfa, comercializada por el laboratorio farmacéutico Serono bajo el nombre comercial Gonal F.


BRITAIN'S BIGGEST EVENING SALE

Evening News

LATE SPECIAL CITY PRICES

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

Figura 4: Portada de un diario londinense de 1978 donde se recoge la noticia del nacimiento de Louis Brown en la portada.

Se ha avanzado aún más y, en 1992, se describió una nueva folitropina con una vida media mayor creada por recombinación genética mediante la unión del extremo final del gen de la cadena beta de la FSH, menor vida media, al final del gen de la hCG, mayor vida media (Fares et ál. 1992). El nombre genérico de esta nueva gonadotropina es corifolitropina alfa (Elonva, MSD) y está disponible comercialmente desde 2010.

La síntesis de agonistas y antagonistas de la GnRH

Un paso más en el avance de los tratamientos de la disfunción reproductiva fue el descubrimiento de la GnRH, "Gonadotropin-releasing hormone", hormona liberadora de gonatropinas (Raju, 1999). La GnRH es un decapéptido que se sintetiza en el núcleo arcuato del hipotálamo. Fue aislada por dos grupos de forma independiente, el de Andrew V. Schally y el de Roger C.L. Guillemin (Figura 5). Ambos investigadores recibieron el premio Nobel de Medicina en 1977 por este descubrimiento.

Existen dos tipos de GnRH, la tipo I y la tipo II. La primera codificada por el brazo corto del cromosoma 8 y la segunda por el

brazo corto del cromosoma 20 (Huirne y Lambalk 2001). La GnRH se une a un receptor de membrana específico acoplado a proteínas G que se encuentra en la célula diana (figura 6). Tras la activación del receptor, la célula hipofisaria liberará gonadotropinas, (FSH y LH) de forma pulsátil al torrente sanguíneo. Esto convierte a la GnRH en un punto de control único, y crucial, del sistema reproductivo (Speroff y Fritz, 2006).

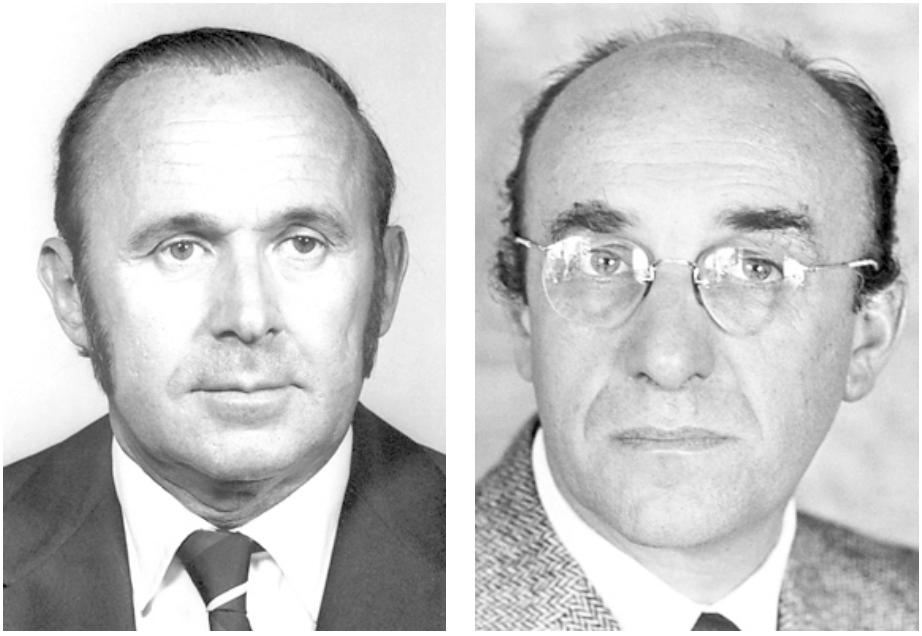


Figura 5: Andrew V. Schally (izquierda) y Roger Gulleim. Premio Nobel compartido de Medicina 1977 por el descubrimiento de la GnRH.

La vida media de la GnRH es de entre 2 y 4 minutos. La estimulación de la GnRH desde el hipotálamo a la hipófisis es pulsátil, con una frecuencia de un pulso entre 1 y 4 horas. Si desaparece esta oscilación en la secreción se ocasiona una primera hipersecreción rápida de LH y FSH seguida de una desensibilización y disminución de la secreción (Speroff y Fritz 2006).

El conocimiento de la secuencia de aminoácidos de la GnRH permitió el desarrollo de agonistas y antagonistas de la GnRH. Un solo cambio en un aminoácido de la secuencia puede alterar la

afinidad del agonista al cambiar la cadena que interactúa con la proteína receptora (Huirne & Lambalk 2001).

En los tratamientos de FIV, la utilización de agonistas y antagonistas de la GnRH ha venido determinada por la necesidad de controlar la aparición de picos prematuros de LH que conllevan a una ovulación. La estimulación ovárica controlada da lugar a un reclutamiento multifolicular acompañado de un aumento exagerado de estradiol circulante que puede inducir la liberación súbita e inesperada de LH y a tener que cancelar el ciclo por

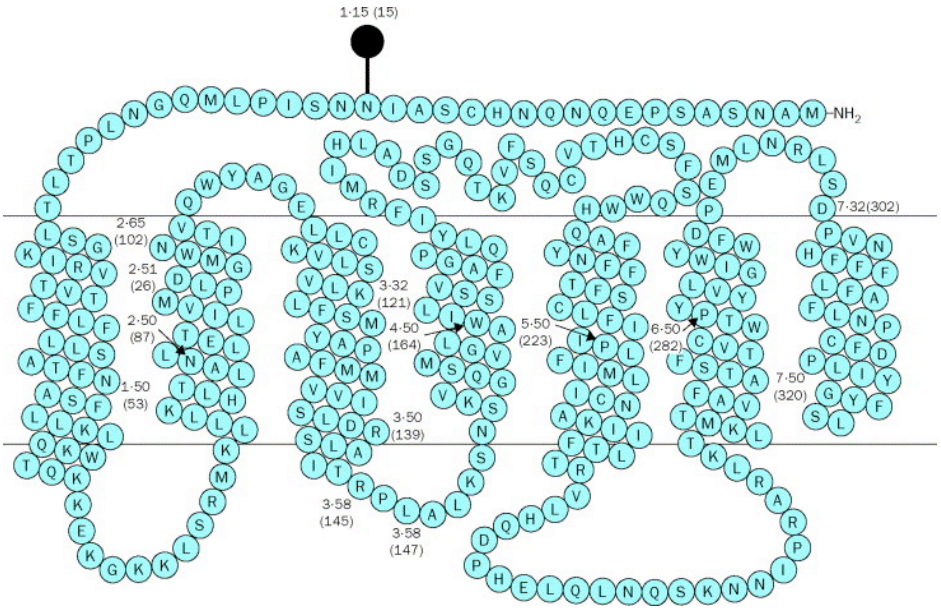


Figura 6: Esquema del receptor de la GnRH. Tomado de: J. A. Huirne and C. B. Lambalk, "Gonadotropin-releasing-hormone-receptor antagonists." *Lancet*, vol. 358, pp. 1793–1803, Nov. 2001.

luteinización precoz. La introducción de los análogos de la GnRH en los ciclos de FIV permitió evitar las cancelaciones por este motivo (Coccia et ál. 2004).

La síntesis de agonistas de la GnRH, requiere de pocos cambios en la estructura de la cadena peptídica de la GnRH (Figura 7). Por ejemplo, con cambios en los enlaces entre los aminoácidos 5-6, 6-7 y 9-10, se pueden sintetizar análogos de la GnRH con propiedades diferentes. Los agonistas producen un efecto inicial estimulante de FSH y LH, denominado "flare up", seguido de una

desensibilización de los gonadotropos y de una regulación a la baja del número de receptores en la membrana celular ocasionando una disminución en la liberación de FSH y LH hasta que la cantidad de ambas en la hipófisis es mínima (Coccia et ál. 2004).

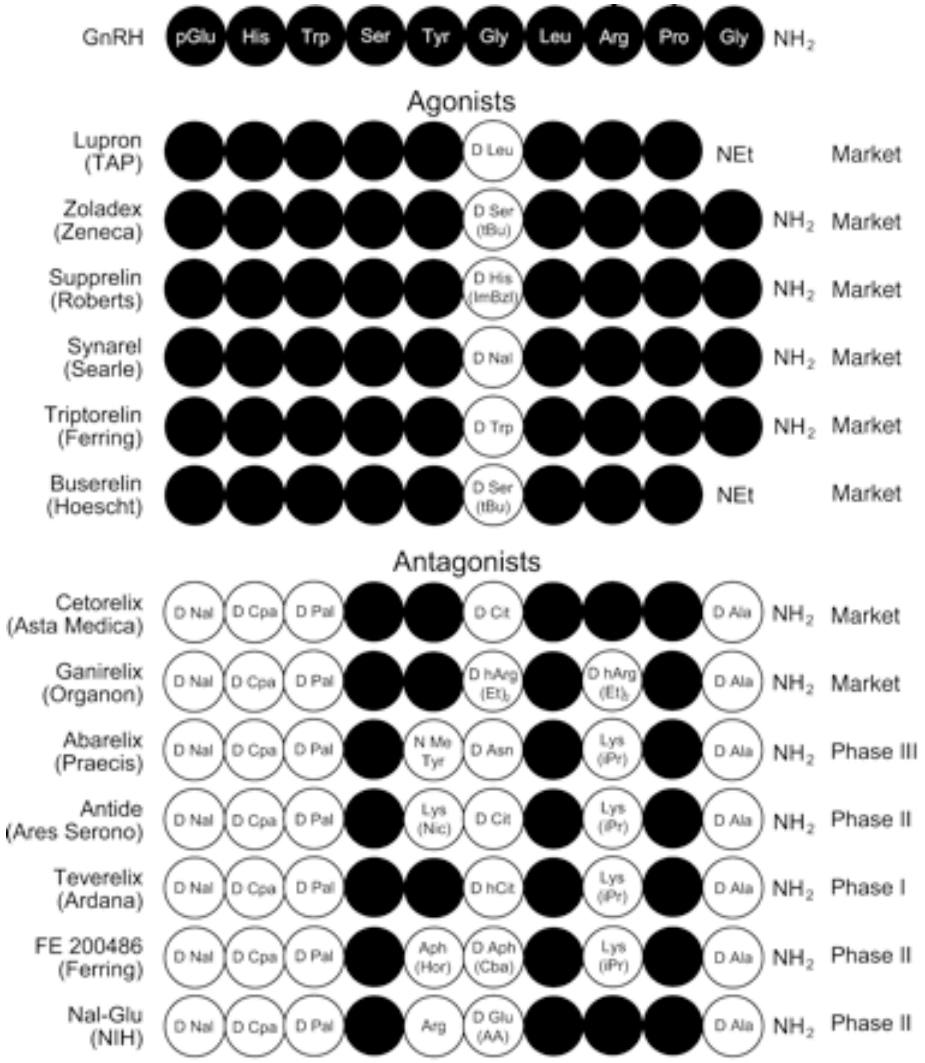


Figura 7: Secuencia de la GnRH y modificaciones realizadas para producir agonistas o antagonistas. Fuente: (Millar:2004)

La sustitución de los aminoácidos en la posición 6 de la cadena de la GnRH da lugar a antagonistas que bloquean de forma inmediata el receptor haciendo que los niveles plasmáticos de FSH y LH disminuyan en horas. La vida media de un antagonista de la GnRH tras una inyección subcutánea oscila entre las 5 y 30 horas. La amplitud y duración de este efecto es dosis dependiente y es completamente reversible en 24-72 horas (Huirne y Lambalk 2001).

Sin embargo, la adenohipófisis sigue conteniendo FSH y LH y mantiene la posibilidad de responder a la GnRH nativa o a un agonista de la misma. Los antagonistas de la GnRH se sintetizan realizando múltiples sustituciones de aminoácidos en la cadena original de la GnRH (Figura 7). Al contrario de lo que ocurrió con los agonistas, se ha tardado más de 30 años en disponer de antagonistas de la GnRH aprobados para uso clínico. La primera generación tenía el inconveniente de producir severos efectos secundarios, en concreto reacciones anafilácticas debido a que ocasionaban una intensa liberación de histamina. La segunda generación de antagonistas también seguía ocasionando efectos adversos y no ha sido hasta la tercera (cetrorélix, ganirélix, degarélix) que se han podido incorporar a los protocolos de los tratamientos de reproducción asistida (Coccia et ál. 2004; Millar 2004).

Como se ha comentado anteriormente, la estimulación ovárica controlada es un procedimiento estándar en los ciclos de IA y FIV que ha hecho posible conseguir un aumento notable en el porcentaje de éxito respecto a los ciclos en los que se no practica dicha estimulación. Sin embargo, el reclutamiento multifolicular ocasiona una elevación mayor del estradiol plasmático que a su vez puede ocasionar que aparezca una liberación de LH cuando aún los folículos no han completado su crecimiento y diferenciación. En lo que respecta a los ciclos de FIV, la luteinización precoz está controlada por la incorporación de los análogos de la GnRH a la estimulación ovárica (Coccia *et ál.*, 2004). Esta forma de actuar no ha sido incorporada a la estimulación ovárica en los ciclos de IA y, sin embargo, se ha calculado que hasta en un 24% de los ciclos de IA han de ser cancelados por luteinización precoz (Gómez-Palomares *et ál.*, 2005). Esto conlleva un evidente estrés psicológico además de un aumento del coste del tratamiento para las pacientes.

Por otra parte, para evitar la aparición del pico prematuro de LH es práctica habitual inducir la ovulación y programar la IA tan pronto como el folículo dominante alcanza los 18 mm de diámetro, independientemente del número o estado del resto de los folículos acompañantes. Este proceder transforma un ciclo que podría haber sido multifolicular en monofolicular y, en consecuencia, disminuye las posibilidades de embarazo, dado que al menos dos folículos maduros (de 16 o más milímetros) son necesarios para conseguir unas tasas de embarazo aceptables (Kaplan et ál. 2002; Nuojua-Huttunen et ál. 1999).

Anteriormente a la aparición de los antagonistas de tercera generación, en los ciclos de FIV en que la frenación de la hipófisis se hacia con agonistas de la GnRH, la ovulación y la maduración final del oocito había que inducirla con la hCG obligatoriamente; con el consiguiente riesgo de inducir un síndrome de hiperestimulación ovárica (SHO). Sin embargo, la posibilidad de provocar la ovulación con agonistas de las GnRH en los ciclos en el que la supresión hipofisaria se lleva a cabo con un antagonista, no hace necesaria el uso de la hCG, ya que es la LH endógena la que induce la maduración del oocito, por lo que el riesgo de SHO en los tratamientos de reproducción asistida ha quedado reducido casi a cero.

Objetivos

Los objetivos de esta tesis son:

1. Estudiar la utilidad de los antagonistas de la GnRH en la programación de los ciclos de inseminación artificial con el fin de controlar la luteinización precoz.

2. Determinar si el uso de un antagonista de la GnRH aumenta las tasas de embarazo en los ciclos de inseminación artificial.

3. Demostrar que la maduración final de los oocitos con un agonista de la GnRH no compromete las tasas de implantación.

4. Aseverar que en los ciclos de donación de oocitos, no ha lugar la cancelación, el "coasting" o el síndrome de hiperestimulación ovárica si se usan los antagonistas de la GnRH para inducir la ovulación.

5. Demostrar que en los ciclos con antagonista de la GnRH, la inducción de la ovulación con un agonista de la GnRH no ocasiona hiperestimulación ovárica en pacientes con ovarios poliquísticos.

OBJETIVO 1

Estudiar la utilidad de los antagonistas de la GnRH en la programación de los ciclos de inseminación artificial con el fin de controlar la luteinización precoz.

Antagonistas de la GnRH en la programación de los ciclos de inseminación artificial.

J.L.Gómez-Palomares, B. Juliá,
B.Acevedo-Martín, M.Martínez-Burgos, E.R.Hernández, E.Ricciarelli

Human Reproduction
Vol.20, No.2 pp. 368–372, 2005

La inclusión de los antagonistas de la GnRH en los tratamientos de IA permite controlar el crecimiento folicular sin el riesgo de luteinización precoz. Si las posibilidades de éxito son mayores al aumentar el número de folículos disponibles presentes el día en el que se programa la inseminación intrauterina y si el factor limitante es la luteinización precoz, el uso del antagonistas permite prolongar el desarrollo folicular de un mayor número de folículos periovulatorios y en consecuencia mejorar las tasas de embarazo.

Para estudiar la utilidad de los antagonistas de la GnRH en los ciclos de inseminación artificial realizamos este estudio, multicéntrico, prospectivo y aleatorizado, en FivMadrid y en el Hospital Severo Ochoa de Leganés. 82 parejas con un rango de edad de entre 18 y 38 años fueron incluidas. En todos los casos, los valores hormonales ováricos, tiroideos y la prolactina debían estar en el rango normal. Además, la cavidad uterina no debía tener alteraciones y las trompas debían ser permeables. Se excluyeron las mujeres diagnosticadas de síndrome de ovario poliquístico y los varones en los que el recuento seminal por capacitación espermática fuera menor a 10 millones por mL.

La estimulación ovárica se llevó a cabo con FSH recombinante (FSHr) previa confirmación del reposo ovárico mediante ecografía realizada entre el tercer y cuarto día del ciclo.

La asignación, secuencial aleatoria de las pacientes se realizó mediante un listado de números aleatorios generado por ordenador.

En el grupo antagonista fueron incluidos 40 pacientes. En este caso la estimulación se inició entre el tercer y cuarto día del ciclo menstrual tras comprobar el reposo ovárico mediante ecografía transvaginal. La dosis de gonadotropina administrada fue de 100 UI/día durante 5 días. En el 6º día de estimulación se realizó una nueva ecografía para ajustar la dosis. Cuando el folículo dominante alcanzó los 16 mm de diámetro o el valor del estradiol plasmático fue mayor a 300 pg/mL se comenzó la administración, del antagonista de la GnRH (Ganirélix, MSD, 0,25 mg/día) que se mantuvo hasta el momento de la inducción de la maduración final oocitaria con hCG.

Posteriormente, el desarrollo folicular se controló mediante una ecografía realizada cada dos días. Cuando el tamaño folicular llegó a los 20 mm, se administraron 5000 UI/im de hCG (hCG Lepori, Farma Lepori). En el caso de que se observaran más de 4 folículos de entre 16 y 20 mm el ciclo era cancelado, con el fin de reducir el riesgo de embarazo múltiple. No se produjo ningún síndrome de hiperestimulación ovárica grave. Uno de los ciclos fue convertido a ciclo de FIV debido a la alta respuesta (8 folículos de más de 16 mm).

En el grupo control, 42 pacientes se sometieron al mismo protocolo de estimulación ovárica, excepto que no se utilizó el antagonista de la GnRH, y la hCG (misma dosis que en el grupo estudio) se administró una vez que el folículo principal alcanzó los 18-20mm. Al igual que en el grupo antagonista, la inseminación fue cancelada si se reclutaron más de cuatro folículos (16 - 20 mm). En este grupo un ciclo se canceló para evitar un embarazo múltiple ya que la ecografía había revelado siete folículos de más de 16 mm.

Tabla 1. Uso de los antagonistas de la GnRH en los ciclos de inseminación artificial. Parámetros basales.

	Antagonista	Control	p
Número de pacientes	40	42	
Edad (años)[a]	33.9 ± 2.6	32.05 ± 3.3	ns
FSH basal (mUI/L)[a]	5.5 ± 2.1	6.1 ± 1.7	ns
LH basal(mUI/L)[a]	4.7 ± 4.2	6.1 ± 4.8	ns
E2 basal(pg/mL)[a]	38.2 ± 15.4	38.3 ± 12.7	ns

[a] Valores expresados como media ± desviación estándar. Ns: No significativo.

Las muestras de semen se obtuvieron mediante masturbación en un frasco estéril y posterior recuperación de los espermatozoides móviles en el laboratorio mediante la técnica de "swim-up". Tras un tiempo de licuación de entre 20 y 30 minutos, la muestra fue diluida con 2 mL de medio Ham F-10 y centrifugada a 600 g durante 5-10 minutos. Después de descatar el sobrenadante, se añadió 0,3 mL de medio universal para FIV (Medio Universal, Medicult) y la mezcla se dejó reposar a temperatura ambiente durante 30 minutos a temperatura ambiente. Los espermatozoides más móviles fueron aislados y utilizados para la inseminación si el número era superior a 10 millones por mL.

Como es habitual en FivMadrid se practicó una sola inseminación por ciclo entre 36 y 38 h tras la administración de la hCG. En ambos grupos se utilizó un catéter Lee, el volumen de semen introducido fue de 0,3 ml.

La fase lútea se suplementó de forma rutinaria (en todos los pacientes, en ambos grupos) con 300 mg/día de progesterona natural micronizada a partir del mismo día de la inseminación.

A las dos semanas se realizó la prueba de embarazo en sangre mediante la determinación de hCG. En el caso de un resultado positivo, se programó una ecografía transvaginal 2 semanas más tarde para objetivar la presencia del latido cardíaco embrionario.

La progesterona se mantuvo hasta la semana 12 de gestación si ésta era evolutiva.

Como puede observar en la Tabla 1 no hubo diferencias estadísticamente significativas en los niveles basales de FSH (5,5 ± 2,1 UI/L vs 6,1 ± 1,7 UI/L), de LH (4.7 ± 4,2 UI/L vs 6,1 ± 4,8 UI/L) o de estradiol (38,2 ± 15,4 ng/mL vs 38,3 ± 12,7 ng/mL) entre el grupo antagonista y el grupo control, respectivamente.

No hubo tampoco diferencias significativas (Tabla 2) en la cantidad total de FSHr utilizada (707 ± 249 frente a 657 ± 194 UI) ni en los niveles de E2 sérico (428 ± 154 pg/ml frente a 598 ± 167 pg/ml) . Sin embargo, el número de folículos maduros (≥16 mm de diámetro) fue significativamente mayor en el grupo de pacientes tratadas con el antagonista de la GnRH que en el grupo control (2.4 ± 1.4 frente a 1.7 ± 1.2, p= 0,02).

Tabla 2. Uso de los antagonistas de la GnRH en los ciclos de inseminación artificial. Estimulación.

	Antagonista	Control	p
Ampollas	1,8 ± 0.7	-	
FSH (unidades)	707 ± 240	657 ± 194	ns
Estimulación (días)	7,6 ± 1.9	6,6 ± 1.8	ns
Folículos ≥16mm	2,4 ± 1.4	1,7 ± 1.2	0.02
Estradiol	428 ± 154	598 ± 167	ns
Swim-up (mill./ml.)	23.4 ± 9.3	19.9 ± 18.4	ns

Todos los valores expresados como media ± desviación estándar. Ns: No significativo.

Como era de esperar, la duración del ciclo se extendió en casi un día más (7,6 ± 1,9 frente a 6,6 ± 1.8 días) en el grupo antagonista, aunque esta diferencia no fue estadísticamente significativa.

Las tasas de embarazo (Tabla 3) se incrementaron significativamente en el grupo de los pacientes que recibieron el antagonista de la GnRH (38%) en comparación con el grupo control (14%). El 93% de los embarazos conseguidos en el grupo antagonista (14/15) y el 100% de los embarazos en grupo control

(6/6) fueron gestaciones únicas. Hubo una única gestación triple que ocurrió en el grupo antagonista.

Tabla 3. Uso de los antagonistas de la GnRH en los ciclos de inseminación artificial. Embarazos

	Antagonista	Control	p
Total	15/39	7/41	0.03
Bioquímicos, % (n)	0 (0)	14 (1/7)	ns
Clínicos, % (n)	38 (15/39)	14 (6/41)	0.01
Abortos, % (n)	0 (0)	0 (0)	ns
Únicos, % (n)	93 (14/15)	100 (6/6)	ns
Múltiples, % (n)	6.6 (1/15)	0 (0/6)	ns

El propósito de este estudio fue el de determinar si la inclusión de un antagonista de la GnRH (Ganirélix), en un protocolo de inseminación artificial con estimulación ovárica controlada, podía aumentar las tasas de embarazo al permitir aumentar el número de folículos disponibles el día de la inseminación intrauterina. Los resultados apoyaban esta hipótesis al comprobarse que en el grupo en el que se había utilizado el antagonista (1,8± 0,7 ampollas) se observó un número mayor folículos maduros (≥16 mm) respecto al grupo control y por tanto una mayor posibilidad de embarazo, (2,4 ± 1,4 folículos vs 1,7 ±1,2 folículos respectivamente). Sin embargo al tener un mayor número de folículos maduros las posibilidades de que ocurriera un embarazo múltiple aumentaron. De hecho, la única gestación triple se produjo en el grupo que recibió el antagonista.

En referencia a los niveles de estradiol durante el ciclo, en el grupo antagonista fueron menores que en el grupo control, aunque esta diferencia no fue significativa. Esto no impidió que se alterara la diferenciación folicular dado que las tasas de embarazo fueron significativamente más altas en el grupo en el que se añadió el antagonista al protocolo de estimulación ovárica. Sin embargo, es preciso remarcar que esto estuvo relacionado, más probablemente,

con el mayor número de folículos disponibles el día de la inseminación que con el uso del antagonista en sí.

Este estudio fue incluido posteriormente en un meta-análisis publicado en 2008 (Kosmas *et ál*. 2008) que coincidía con nosotros al concluir que dejar crecer algo más los folículos en un ciclo de inseminación así como inhibir los picos prematuros de LH aumentaba claramente las posibilidades de conseguir un embarazo.

Human Reproduction Vol.20, No.2 pp. 368–372, 2005 doi:10.1093/humrep/deh602
Advance Access publication November 26, 2004.

Timing ovulation for intrauterine insemination with a GnRH antagonist

J.L.Gómez-Palomares[1], B.Juliá[2], B.Acevedo-Martín[1], M.Martínez-Burgos[1], E.R.Hernández[1,3] and E.Ricciarelli[1]

[1]Clínica de Medicina de la Reproducción y Ginecología 'FIVMadrid', Madrid and [2]Unidad de Reproducción, Hospital 'Severo Ochoa', Madrid, Spain

[3]To whom correspondence should be addressed at: Clínica de Medicina de la Reproducción y Ginecología 'FIV Madrid', C/Álvarez de Baena 4 bajo, 28006 Madrid, Spain. E-mail: ehernandezm@fivmadrid.es

BACKGROUND: We aimed to assess the efficacy of a GnRH antagonist in intrauterine insemination (IUI) cycles to increase number of mature ovulatory follicles and pregnancy rates. METHODS: Prospective randomized study. Women (18–38 years old) with primary/secondary infertility were included. Eighty-two patients were randomly assigned to controlled ovarian stimulation (COS) consisting of rFSH + GnRH antagonist or rFSH alone. RESULTS: A non-significant increase in the total amount of rFSH was seen in the GnRH antagonist group (707 ± 240 IU) with respect to the control group (657 ± 194 IU). The number of mature follicles (≥16 mm) was significantly higher in the GnRH antagonist group than in the control group (2.4 ± 1.4 versus 1.7 ± 1.2, $P < 0.05$). Pregnancy rates were significantly increased in the group of patients receiving the GnRH antagonist (38%) compared to the control group (14%). The only non-single pregnancy (triplets) occurred in the antagonist group. CONCLUSIONS: In this preliminary study, adding the GnRH antagonist to the COS protocol for IUI cycles significantly increased pregnancy rates. Nevertheless, these results may not be associated directly with the antagonist itself but with the fact that more mature ovulatory follicles are present by the day of the hCG. Finally, the risk for multiple gestations needs to be carefully evaluated.

Key words: GnRH antagonist/intrauterine insemination/pregnancy rates

Introduction

Controlled ovarian stimulation (COS) is a standard procedure in intrauterine insemination (IUI) that has resulted in a significant increase in pregnancy rates with respect to rates in non-stimulated IUI cycles (Nuojua-Huttunen *et al*., 1999; Dickey *et al*., 2002; Duran *et al*., 2002; Houmard *et al*., 2002; Kaplan *et al*., 2002). Nevertheless, multifollicular recruitment during COS can bring about a sudden increase in estradiol (E_2) serum levels that is enough to induce an LH surge while follicular growth is still in progress. Moreover, it has been calculated that 24% of IUI cycles suffer undesired premature luteinization (Ragni *et al*., 2004) and this can result in IUI procedure cancellation. Obviously this represents economic and psychological stress for the patients. Thus, to avoid the risk of unexpected premature follicular luteinization, standard procedure induces ovulation as soon as the leading follicle has reached the 18 mm boundary, independently of the number and developmental status of the other recruited follicles (Frydman *et al*., 1991; Duran *et al*., 2002). This will necessarily change the IUI cycle from multifollicular to monofollicular and, consequently, decrease the chances of gestation, since at least two mature follicles (≥16 mm) are needed to attain an acceptable pregnancy rate in IUI (Nuojua-Huttunen *et al*., 1999; Dickey *et al*., 2002; Duran *et al*., 2002; Houmard *et al*., 2002; Kaplan *et al*., 2002).

The inclusion of the GnRH antagonist in assisted reproduction techniques allows ovulation to be postponed since the antagonist will suppress gonadotrophin release, block the possibility of premature LH surges, and, consequently, premature luteinization of the follicles (Frydman *et al*., 1991; Diedrich *et al*., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker *et al*., 2001). Furthermore, this control of untimely LH release has been extensively confirmed and demonstrated to successfully protect follicular development against unexpected luteinization in IVF (Diedrich *et al*., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker *et al*., 2001; Ricciarelli *et al*., 2003; Acevedo-Martin *et al*., 2004).

With this in mind, we thought that if pregnancy rates in IUI were related to the number of mature follicles present on the day hCG was indicated (Nuojua-Huttunen *et al*., 1999; Dickey *et al*., 2002; Duran *et al*., 2002; Houmard *et al*., 2002; Kaplan *et al*., 2002), and if the limiting factor for follicular development was premature luteinization, why not control unexpected LH surges during the COS–IUI cycles

with an antagonist of the GnRH? This would: (i) maintain steady growth up to >20 mm in diameter among the recruited follicles; (ii) allow us to time ovulation in a synchronized manner with more than one mature follicle and, hopefully, increase pregnancy rates.

Materials and methods

Study design

This study was a multicentre, prospective, randomized study performed from January to June, 2003, in 82 infertile couples to assess the efficacy of GnRH antagonist in COS combined with homologous IUI.

The GnRH antagonist is already routinely used for IVF in our centres, and the patients were recruited following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on human research.

Inclusion criteria

The main inclusion criteria in women were age between 18 and 38 years, regular menstrual cycle, primary or secondary infertility lasting for ≥12 months, body mass index between 19 and 25 kg/m^2, normal prolactin levels, normal thyroid function, normal uterine cavity and bilateral tubal patency assessed by hysterosalpingography and/or laparoscopy.

Women with hormone values outside the reference range by day 3–4 of their menstrual period (FSH levels >10 mIU/l) and with polycystic ovarian syndrome were excluded from this study.

Semen analysis was performed at least twice and IUI was carried out if the total motile spermatozoid swim-up count was >10 × 10^6/ml.

Hormonal treatment

COS was performed in both groups with recombinant FSH (rFSH, Puregon; Organon Inc., Spain). Patients included in this study underwent an ultrasound scan between the third and fourth day of their menstrual cycle and were subsequently randomized by a computer-generated random listing into two groups. The study was performed in two centres. Patients were randomly assigned by a computer-generated list in the order of their enrolment.

GnRH antagonist group

Forty patients (30 patients from the first centre and 10 from the second centre) were subjected to a COS protocol in which the LH surge was suppressed with the GnRH antagonist ganirelix acetate (Orgalutran; Organon Inc., Spain), as follows. On the third or fourth day of their menstrual period, the patients were examined by ultrasound to ensure ovarian quiescence. A fixed dose of 100 IU/per day of rFSH was then given to induce follicle recruitment for 5 days. At day 6 of ovarian stimulation, an ultrasound was performed to adjust FSH dose, if necessary. When the recruited follicles were ≥16 mm or E_2 levels were >300 pg/ml, 0.25 mg of ganirelix was subcutaneously injected daily following the manufacturer's guidelines until the day hCG was given. Subsequently, follicular development was controlled by sequential ultrasound scans every 2 days. When adequate ovarian response was observed (follicles =20 mm), 5000 IU/i.m. hCG (hCG-Lepori; Farma-Lepori, Spain) was administered. IUI was cancelled if more than four follicles (>16–20 mm) were present, in order to reduce the risk of multiple pregnancy. No severe ovarian hyperstimulation syndrome occurred. One cycle was converted to an IVF cycle due to excessive ovarian response (eight follicles ≥16 mm).

Control group

Forty-two patients (31 from the first centre and 11 from the second centre) were subjected to the same COS protocol previously described except that the GnRH antagonist (Ganirelix) was not used and hCG (5000 IU) was administered once the leading follicle reached the 18–20 mm boundary, on ultrasound examination. As in the other group, IUI was cancelled if more than four follicles (>16–20 mm) were present. One cycle was cancelled in order to avoid a multiple pregnancy since ultrasound had revealed seven follicles ≥16 mm.

Semen preparation

Semen specimens were obtained by masturbation into a sterile jar after 2–3 days of abstinence and a few hours before the scheduled insemination. After liquefaction for ~20–30 min, the specimen was mixed and diluted with 2 ml of Ham's F-10 medium (Gibco, UK) The mixture was centrifuged at 600*g* for 5–10 min, and the supernatant discarded; 0.3 ml of IVF Universal medium (Medicult, Denmark) was added and the mixture incubated for 30–45 min at room temperature. After the swim-up, the most active motile sperm were isolated by aspiration and used for insemination.

IUI procedure and luteal phase support

A single insemination was performed, 36–38 h post-hCG, in both groups using a Lee catheter (Vygon, France) inserted through the cervix. A limited insemination volume (0.3 ml) was delivered into the uterine cavity and bed rest was maintained for 10 min after IUI.

The luteal phase was routinely supplemented (in all patients in both groups) with 300 mg/day/vaginally of natural micronized progesterone (Utrogestan; Seid, Spain) starting after the IUI procedure on the same day.

Sixteen days after the insemination, an hCG assay (HCG; BioMerieux, France) was performed. If the assay was positive, transvaginal ultrasonography was scheduled for 2 weeks later. The concurrency of a positive hCG and embryo(s) with a positive heart beat (seen by ultrasound) was defined as a clinical pregnancy—otherwise the positive hCG test was considered to be a biochemical pregnancy. Progesterone was maintained until the 12th week of pregnancy if a clinical pregnancy was evident.

Hormonal determination

FSH, LH, E_2 and hCG serum levels were determined with a commercial enzyme immunoassay kit (Vidas; Biomérieux, Spain). Blood samples were collected on the third or fourth day of the cycle (E_2, LH and FSH) and before given hCG (E_2).

Statistical analysis

Data were expressed as the mean ± SD. Continuous variables were compared with Student's *t*-test. The χ^2-test and Fisher test were used to compare clinical outcome between the two groups. The analysis was carried out using the statistical package for social sciences (SPSS Inc., USA). $P < 0.05$ was considered significant.

Results

To determine the impact that inclusion of GnRH antagonist in COS–IUI cycles may have on pregnancy rates, 82 patients were randomly divided as described in Materials and methods.

Table I. Timing ovulation for intrauterine insemination with a GnRH antagonist

	GnRH antagonist	Control	*P*
No. of patients	40	42	
Age (years)[a]	33.9 ± 2.6	32.05 ± 3.3	NS
Basal FSH (mIU/l)[a]	5.5 ± 2.1	6.1 ± 1.7	NS
Basal LH (mIU/l)[a]	4.7 ± 4.2	6.1 ± 4.8	NS
Basal estradiol (pg/ml)[a]	38.2 ± 15.4	38.3 ± 12.7	NS
Main causes of infertility (*n*)			
Unknown	30	28	NS
Anovulation	10	14	NS
Parity (*n*)			
Primary infertility	36	39	NS
Secondary infertility	4	3	NS

[a]Values are means ± SD.
NS = not significant.

Table II. Timing ovulation for intrauterine insemination with a GnRH antagonist

	GnRH antagonist	Control	*P*
No. of ampoules	1.8 ± 0.7	–	
FSH (total IU)	707 ± 240	657 ± 194	NS
Days of stimulation	7.6 ± 1.9	6.6 ± 1.8	NS
Follicles ≥16 mm	2.4 ± 1.4	1.7 ± 1.2	0.02
Estradiol	428 ± 154	598 ± 167	NS
Swim-up sperm ($\times 10^6$/ml)	23.4 ± 9.3	19.9 ± 18.4	NS

All values are means ± SD.
NS = not significant.

As seen in Table I, no significant differences in basal serum levels (third and fourth days of their menstrual cycle) for FSH (5.5 ± 2.1 versus 6.1 ± 1.7), LH (4.7 ± 4.2 versus 6.1 ± 4.8) or E_2 (38.2 ± 15.4 versus 38.3 ± 12.7) were noted between the GnRH antagonist-treated group and the control group.

No significant differences in the total amount of rFSH (707 ± 240 versus 657 ± 194 total IU) and E_2 serum levels (428 ± 154 pg/ml versus 598 ± 167 pg/ml) were seen between the GnRH antagonist and the control groups (Table II).

Nevertheless, the number of mature follicles (≥16 mm in diameter) determined by ultrasound before beginning hCG was significantly higher in patients receiving GnRH antagonist than in the control group (2.4 ± 1.4 versus 1.7 ± 1.2, $P < 0.05$). As expected, cycle length was extended to almost 1 day more (7.6 ± 1.9 versus 6.6 ± 1.8 days) in the GnRH antagonist group than in the control group, although this difference was not statistically significant.

Pregnancy rates were significantly increased in the group of patients receiving the GnRH antagonist (38%) compared to the control group (14%). To date, except for one biochemical pregnancy in the control group, all the other pregnancies are ongoing. Furthermore, 93% of the pregnancies in the GnRH antagonist group (14/15) and 100% of the pregnancies in the control group (6/6) were single gestations. The only non-single pregnancy (triplets) occurred in the antagonist group.

Discussion

The purpose of this preliminary study was to determine if the inclusion of a GnRH antagonist (ganirelix acetate) in COS–IUI protocols could significantly increase the number of mature follicles and, by extension, improve pregnancy rates, since these two parameters are known to be positively correlated (Nuojua-Huttunen *et al*, 1999; Dickey *et al*., 2002; Duran *et al*., 2002; Houmard *et al*., 2002; Kaplan *et al*., 2002).

The rationale for this hypothesis was based on the capacity of the GnRH antagonist to rapidly inhibit LH release by the gonadotrophs and thereby control and avoid premature luteinization in IVF (Frydman *et al*., 1991; Diedrich *et al*., 1994; Reissmann *et al*., 1995; Olivennes *et al*., 2002). We thought that the same mechanism could be used in COS–IUI advantageously to avoid premature luteinization of the leading follicle(s) and to expand follicular development so that ovulation could be induced once follicle size was between 19 and 20 mm. Furthermore, since COS generally develops a cohort of follicles, avoiding premature luteinization with ganirelix acetate would increase the number of ovulatory follicles available for the IUI procedure and, hopefully, increase pregnancy rates (Nuojua-Huttunen *et al*, 1999; Dickey *et al*., 2002; Duran *et al*., 2002; Houmard *et al*., 2002; Kaplan *et al*., 2002).

Our results support this hypothesis since the inclusion of the GnRH antagonist (1.8 ± 0.7 ampoules) in the COS–IUI protocol significantly ($P < 0.05$) increased the number of mature follicles (≥16 mm) in the GnRH antagonist group with respect to the control group (2.4 ± 1.4 versus 1.7 ± 1.2 respectively). The total amount of FSH used by patients receiving GnRH antagonist (707 ± 240 total IU) was slightly greater than in the controls (657 ± 194 total units), but this difference was not significant (Table II).

Nevertheless, we were concerned by the increased cost of including the GnRH antagonist in the IUI protocols. We calculated that including this treatment would increase the cost of each cycle by a minimum of €100 and a maximum of €150. In return by using a GnRH antagonist that will significantly reduce the risk of cycle cancellation and the associated emotional stress to the patients.

We were aware of the risk that increasing the number of mature follicles could also increase multiple pregnancy rates (Dickey *et al*., 2001; Khalil *et al*., 2001; Tur *et al*., 2001), and, in fact, the only multiple gestation in this study was in the group treated with GnRH antagonist (Table III). For this reason, IUI was cancelled whenever a patient had more than four 16–20 mm follicles, regardless of how many follicles were <15 mm (Claman, 2004; Osuna *et al*., 2004). Several

Table III. Timing ovulation for intrauterine insemination with a GnRH antagonist

	GnRH antagonist	Control	*P*
Pregnancies			
Total	15/39	7/41	0.03
Biochemical, % (*n*)	0 (0)	14 (1/7)	NS
Clinical, % (*n*)	38 (15/39)	14 (6/41)	0.01
Miscarriages, % (*n*)	0 (0)	0 (0)	NS
Singletons, % (*n*)	93 (14/15)	100 (6/6)	NS
Multiple, % (*n*)	6.6 (1/15)	0 (0/6)	NS

NS = not significant.

publications have indicated that the risk of multiple pregnancies rises with the number of 15 mm follicles (Tur *et al*., 2001; Dickey *et al*., 2001; Khalil *et al*., 2001; Ghosh *et al*., 2003) and some of the follicles we classified as 15 mm could actually have measured 16 mm (given the difficulties of distinguishing between a 16 or 15 mm follicle by ultrasound). However, our clinic's historical average for multiple gestations using the criterion of ignoring the number of <15 mm follicles when deciding IUI is 20% (only twins, no triplets), so we followed the same rationale in this study and only one couple in the GnRH antagonist group had a multiple pregnancy.

As also described previously in IVF /GnRH antagonist cycles (Diedrich *et al*., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker *et al*., 2001; Ricciarelli *et al*., 2003; Acevedo-Martin *et al*., 2004), E_2 serum levels decreased in the GnRH antagonist–IUI group in respect to the control group (428 ± 154 versus 598 ± 167 respectively), although not significantly. This decrease did not affect oocyte differentiation (Hernández, 2000; Tur *et al*., 2001; Ricciarelli *et al*., 2003; Acevedo-Martin *et al*., 2004) since pregnancy rates were significantly increased in patients receiving the GnRH antagonist with respect to the controls (38 versus 14%). Furthermore, at the time of writing, all the pregnancies are ongoing in the GnRH antagonist group and there have been no miscarriages (Table III). However, it must be remembered that the increases in the pregnancy rate are probably not related to the antagonist itself but to the fact that the use of an antagonist gives the clinician more time to produce more ovulatory follicles that can give rise to a pregnancy.

Although Kolibianakis *et al*. (2003) report that patients receiving the GnRH antagonist during IVF cycles seem to present a dysfunctional endometrium, Ragni *et al*. (2001) have demonstrated that the luteal phase profile (progesterone concentration) in COS–IUI/GnRH antagonist cycles seems to be unaffected in IUI cycles treated with recombinant FSH in combination with GnRH antagonist. Nevertheless, to avoid a hypothetical effect of the antagonist on the function of the corpus luteum, we routinely supplemented the luteal phase with natural micronized progesterone in all the IUI procedures.

In conclusion, the addition of the GnRH antagonist to COS–IUI cycles significantly increased pregnancy rates in our patients. Since this increase seems to be related to the number of follicles recruited, clinicians should balance this benefit against the risk of multiple gestation in IUI.

Finally, because only a relatively small number of patients has been analysed, the results of this study are only preliminary. However, in our opinion some immediate emotional and clinical benefits (lower cancellation rates, avoidance of premature luteinization) can be obtained using the GnRH antagonist in the COS–IUI cycles.

References

Acevedo-Martin B, Sanchez M, Gomez-Palomares JL, Cuadros J, Ricciarelli E and Hernández ER (2004) LH-supplementation increased pregnancy rates in gonadotropin-releasing hormone antagonist donor cycles. Fertil Steril 82,343–347.

Claman P (2004) Simplifying superovulation and intrauterine insemination treatment: evidence and clinical decision making. Fertil Steril 82, 32–33.

Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH and Pyrzak R (2001) Relationship of follicle numbers and estradiol levels to multiple implantation in 3,608 intrauterine insemination cycles. Fertil Steril 75, 69–78.

Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH and Pyrzak R (2002) Effect of diagnosis, age, sperm quality, and number of preovulatory follicles on the outcome of multiple cycles of clomiphene citrate-intrauterine insemination. Fertil Steril 78,1088–1095.

Diedrich K, Diedrich C, Santos E, Zoll C, al-Hasani S, Reissmann T, Krebs D and Klingmuller D (1994) Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. Hum Reprod 9,788–791.

Duran HE, Morshedi M, Kruger T and Oehninger S (2002) Intrauterine insemination: a systematic review on determinants of success. Hum Reprod Update 8,373–384.

European and Middle East Orgalutran Study Group (2001) Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. Hum Reprod 16, 644–651.

Fluker M, Grifo J, Leader A, Levy M, Meldrum D, Muasher SJ, Rinehart J, Rosenwaks Z, Scott RT, Jr, Schoolcraft W and Shapiro DB (2001) North American Ganirelix Study Group Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. Fertil Steril 75,38–45.

Frydman R, Cornel C, de Ziegler D, Taieb J, Spitz IM and Bouchard P (1991) Prevention of premature luteinizing hormone and progesterone rise with a gonadotropin-releasing hormone antagonist, Nal-Glu, in controlled ovarian hyperstimulation. Fertil Steril 56,923–927.

Ghosh C, Buck G, Priore R, Wacktawski-Wende J and Severino M (2003) Follicular response and pregnancy among infertile women undergoing ovulation induction and intrauterine insemination. Fertil Steril 80, 328–335.

Hernández ER (2000) Embryo implantation and GnRH antagonists: embryo implantation, the Rubicon for GnRH antagonists. Hum Reprod 15, 1211–1216.

Houmard BS, Juang MP, Soules MR and Fujimoto VY (2002) Factors influencing pregnancy rates with a combined clomiphene citrate/gonadotropin protocol for non-assisted reproductive technology fertility treatment. Fertil Steril 77,384–386.

Kaplan PF, Katz SL, Thompson AK and Freund RD (2002) Cycle fecundity in controlled ovarian hyperstimulation and intrauterine insemination. Influence of the number of mature follicles at hCG administration. J Reprod Med 47,35–39.

Khalil MR, Rasmussen PE, Erb K, Laursen SB, Rex S and Westergaard LG (2001) Homologous intrauterine insemination. An evaluation of prognostic factors based on a review of 2473 cycles.Acta Obstet Gynecol Scand 80,74–81.

Kolibianakis EM, Bourgain C, Platteau P, Albano C, Van Steirteghem AC and Devroey P (2003) Abnormal endometrial development occurs during the luteal phase of non supplemented donor cycles treated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. Fertil Steril 80,464–466.

Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L and Martikainen H (1999) Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 14,698–703.

Olivennes F, Cunha-Filho JS, Fanchin R, Bouchard P and Frydman R (2002) The use of GnRH antagonists in ovarian stimulation. Hum Reprod Update 8,279–290.

Osuna C, Matorras R, Pijoan JI and Rodríguez-Escudero J (2004) One versus two inseminations per cycle in intrauterine insemination with sperm from patients' husbands: a systematic review of the literature. Fertil Steril 82, 17–24.

Ragni G, Vegetti W, Baroni E, Colombo M, Arnoldi M, Lombroso G and Crosignani PG (2001) Comparison of luteal phase profile in gonadotrophin stimulated cycles with or without a gonadotrophin-releasing hormone antagonist. Hum Reprod 16,2258–2262.

Ragni G, Somigliana E and Vegetti W (2004) Timing of intrauterine insemination: where are we? Fertil Steril 82,25–26.

OBJETIVO 2

Determinar si el uso de un antagonista de la GnRH aumenta las tasas de embarazo en los ciclos de inseminación artificial.

Reclutamiento multifolicular en combinación con el uso de un antagonista de la GnRH en los ciclos de inseminación artificial.

Jose Luis Gómez-Palomares, Belén Acevedo-Martín, Marian Chávez, Mª Ángeles Manzanares, Elisabetta Ricciarelli, y Eleuterio R. Hernández

Fertil Steril. 2008 Mar;89(3):620-4

En relación al uso de antagonistas de la GnRH en inseminación artificial, a diferencia de otros autores en los que la principal preocupación ha sido el evitar la luteinización prematura (Ragni et ál. 2006; Matorras et ál. 2006) en nuestro caso la intención fue la de aumentar el reclutamiento folicular (Gómez-Palomares *et ál.*, 2005). Nuestra hipótesis es que el efecto beneficioso de la inclusión de los antagonistas en este tipo de tratamientos es el resultado de un mayor número de folículos ≥18 mm presentes, y no del antagonista en sí, porque las tasas de embarazo se relacionan con el número de folículos maduros presentes en el momento de la administración de la hCG (Gómez-Palomares *et ál.*, 2005; Nuojua-Huttunen *et ál.*, 1999).

Table 1. Reclutamiento multifolicular en combinación con el uso de un antagonista de la GnRH en los ciclos de IA. Parámetros basales.

	Antagonista	Control
Número de pacientes	184	183
Edad (años)	32,89 ± 2,5	32,05 ± 3,9
Índice de masa corporal	22,9 ± 1,7	23,4 ± 2,2
FSH basal (mIU/L)	6,5 ± 2,3	6,6 ± 2,2
LH basal (mIU/L)	5,3 ± 3,6	5,07 ± 3,3
Estradiol basal (pg/mL)	39,8 ± 22,1	48,7 ± 30,6

Todos los valores han sido expresados como media desviación estándar. En ninguno de los parámetros mostrados hubo diferencias significativas entre los dos grupos.

Tabla 2. Reclutamiento multifolicular en combinación con el uso de un antagonista de la GnRH en los ciclos de IA. Estimulaciones y cancelaciones

	Antagonista	Control	p
Ampollas [a]	2 ± 1.2	-	
FSH (unidades totales) [a]	698.1 ± 310	629.5 ± 358	ns
Días de tratamiento [a]	7.8 ± 2.3	6.9 ± 3.3	ns
Folículos ≥18mm [a]	2.4 ± 1.3	1.3 ± 1.09	<0.05
Estradiol [a]	550 ± 172	631 ± 205	ns
Swim-up (mill./ml.) [a]	25 ± 15.1	31.5 ± 20.8	ns
Cancelaciones totales, n (%)	8 (4.3)	13 (7)	ns
Cancelaciones por riesgo de embarazo múltiple	6 (3.2)	4 (2.1)	ns
Cancelaciones por ovulación	2 (1.08)	9 (5)	<0.05
Síndrome de hiperestimulación	0	0	ns

[a] Media ± desviación estándar. Ns: No significativo.

Con el fin de determinar si el uso de los antagonistas de la GnRH tenía utilidad en los ciclos mono/multifoliculares realizamos un segundo estudio, prospectivo y aleatorizado en 367 parejas con disfunción reproductiva primaria o secundaria de al menos 1 año de duración. Los criterios de inclusión fueron: edad entre 18 y 39 años, índice de masa corporal entre 18 y 28 kg/m^2, permeabilidad tubárica bilateral comprobada, prolactina y hormonas tiroideas en el rango normal. Sólo se incluyeron las pacientes que realizaban su primer ciclo de tratamiento. Fueron criterios de exclusión la determinación de FSH, LH o Estradiol (E2) realizadas en el día 3º, 4º del ciclo menstrual fuera del rango normal, la existencia de endometriosis o de un síndrome de ovario poliquístico. En todos los casos, el recuento de espermatozoides realizado tras la recuperación espermática fue mayor a 10 millones por mL.

En ambos grupos la estimulación ovárica (183 mujeres en el grupo control y 184 en el grupo antagonista) se inició con FSH

recombinante (75-150 UI/día) administrada durante 5 días comenzando entre los días 3er y 4º del ciclo. A partir de ese punto se continúo con un descenso paulatino de las dosis de gonadotropinas hasta alcanzar un tamaño folicular entre 18-20 mm, momento en el que se indujo la ovulación y se programó la inseminación artificial. En el grupo estudio se añadió un antagonista de la GnRH (cetrorélix), 0,25 UI día a partir del momento en el que el folículo alcanzó los 16 mm de diámetro, este fármaco se mantuvo hasta que el tamaño del folículo dominante llegó a los 18-20 mm.

Como se evidencia en la Tabla 1, no hubo diferencias significativas entre ambos grupos en cuanto a la edad, índice de masa corporal u hormonas basales.

Aunque la duración de la estimulación (7,8 ± 2,3 frente a 6,9 ± 3,3 días) y la cantidad total de FSH utilizada (698,1 ± 310 frente a 629 ± 358 unidades internacionales) fue mayor en el grupo antagonista, como se aprecia en la Tabla 2, esta diferencia no fue significativa. Sin embargo el número de folículos reclutados ≥18 mm fue mayor en el grupo antagonista (2,4 ± 1,3) frente al grupo control (1,3 ± 1,09), siendo esta diferencia significativa ($p<0,05$).

De los 367 ciclos iniciados, 21 (5,7%) fueron cancelados (8 en el grupo antagonista y 13 en el grupo control (Tabla 2). Ninguna de las pacientes desarrolló un síndrome de hiperestimulación ovárica.

En cuanto a los embarazos (Tabla 3), el 23% de las pacientes en las que se utilizó el antagonista y el 11% de las incluidas en el grupo control tuvieron una gestación clínica, referida como aquella en la que se consiguió confirmar la presencia de un saco gestacional mediante ecografía. Esta diferencia fue estadísticamente significativa. No se encontraron diferencias en el porcentaje de gestaciones gemelares o abortos. Hubo única gestación triple que ocurrió en el grupo estudio. Si la inseminación se llevó a cabo con sólo un folículo de 18 o más milímetros no hubo

diferencias en cuanto al porcentaje de embarazos conseguidos (16,8% en el grupo antagonista y 14,1% en el grupo control) (Tabla 4).

Tabla 3. Reclutamiento multifolicular en combinación con el uso de un antagonista de la GnRH en los ciclos de IA. Embarazos.

	Antagonista	Control	p
Gestaciones clínicas	23 (42/176)	11 (19/170)	<0,05
Gestaciones únicas	90 (38/42)	73,6 (14/19)	ns
Gestaciones gemelares	4 (2/42)	15,7 (3/19)	ns
Gestaciones triples	2,3 (1/42)	0 (0/19)	ns
Abortos	9,5 (4/42)	10,5 (2/19)	ns

Todos los valores han sido expresados como % (n). Ns= no significativo.

Este estudio demostró que la conjunción de un ciclo multifolicular y la adición de un antagonista de la GnRH en un ciclo de inseminación artificial son la causa del aumento de las posibilidades de quedar gestante. Estos resultados coincidían con los presentados en nuestro trabajo anterior (Gómez-Palomares et ál. , 2005) y corroborado con el presentado por otros investigadores (Ragni et ál. 2004; Allegra et ál. 2007) y parecen reflejar que la inclusión de lo antagonistas de la GnRH en los ciclos de inseminación artificial puede ser una estrategia útil, no sólo ya para evitar la luteinización precoz sino para aumentar las tasas de embarazo. Sin embargo, uno de los hallazgos de este estudio es que

Table 4. Reclutamiento multifolicular en combinación con el uso de un antagonista de la GnRH en ciclos de IA. Tasas de embarazo en relación al número de folículos.

	Antagonista	Control	p
Ciclos monofoliculares	47.1 (83/176)	45.8 (78/170)	ns
Embarazos con 1 folliculo ≥18 mm	16.8 (14/83)	14.1 (13/92)	ns
Embarazos con ≥2 folliculos ≥18 mm	33.3 (31/93)	8.9 (7/78)	<0.05

Todos los datos expresados como % (n). Ns: No significativo.

si el ciclo era multifolicular sí aumentaban las posibilidades de conseguir un embarazo, pero, cuando el ciclo se era monofolicular, no había ninguna ventaja en administrar el antagonista (Tabla 4).

La existencia de un mayor número de folículos disponibles el día en el que se administró la hCG acarreaba también el riesgo bien de desarrollar un síndrome de hiperestimulación ovárica, hecho que no ocurrió en ninguna de las pacientes, bien de desarrollar un embarazo múltiple (paradójicamente hubo más gestaciones gemelares en el grupo control aunque sí se produjo una gestación triple en el grupo antagonista).

En resumen, este estudio demostró que la inclusión de los antagonistas de la GnRH en los ciclos de inseminación artificial sólo es beneficiosa en el caso de que el ciclo sea multifolicular.

Multifollicular recruitment in combination with gonadotropin-releasing hormone antagonist increased pregnancy rates in intrauterine insemination cycles

Jose Luis Gómez-Palomares, M.D., Belén Acevedo-Martín, M.D., Marián Chávez, M.D., M[a] Angeles Manzanares, M.D., Elisabetta Ricciarelli, M.D., and Eleuterio R. Hernández, M.D., Ph.D.

Clìnica de Medicina de la Reproducción y Ginecologìa "FivMadrid," Madrid, Spain

Objective: To determine whether including a GnRH antagonist in controlled ovarian stimulation–intrauterine insemination cycles would increase pregnancy rates.
Design: Prospective randomized study.
Setting: Private reproductive medicine clinic in Spain.
Patient(s): Three hundred sixty-seven women with primary or secondary infertility.
Intervention(s): Patients were randomly assigned to controlled ovarian stimulation with recombinant FSH (75–150 IU/d) alone (controls, n = 183) or with recombinant FSH (75–150 IU/d) + the GnRH antagonist (0.25 mg/d), initiated when the recruited follicles were ≥16 mm (n = 184). A single insemination was performed, 36–38 hours after hCG (5,000 IU, IM), in both groups.
Main Outcome Measure(s): Follicular recruitment, pregnancy rates.
Result(s): Numbers of mature follicles (2.4 ± 1.3 vs. 1.3 ± 1.09) and clinical pregnancy rates (23% vs. 11%) were statistically significantly higher in patients who were treated with GnRH antagonist than in those who were in the control group. The pregnancy rate was only higher in the antagonist group if more than one follicle sized ≥18 mm was present on the day that the hCG was given. A similar number of twin pregnancies occurred in both groups: two in the antagonist group and three in the control group. The antagonist group also had one triplet gestation.
Conclusion(s): Adding GnRH antagonist to controlled ovarian stimulation–intrauterine insemination cycles significantly increases pregnancy rates in multifollicular, but not monofollicular, cycles. (Fertil Steril® 2008; 89:620–4. ©2008 by American Society for Reproductive Medicine.)

Key Words: Ovarian stimulation, GnRH antagonist, IUI, pregnancy rates

To the best of our knowledge, the work of Olivennes et al. (1) was the first study that addressed the use of the GnRH antagonist to control unexpected premature luteinization in controlled ovarian stimulation (COS)–intrauterine insemination (IUI) cycles. Those investigators used a GnRH antagonist that controlled LH release very effectively (2–5).

The results of that study (1) and those of other investigators (6–9) pursuing the same idea found that GnRH antagonists were very useful agents for controlling unexpected luteinization in IUI; however, the studies did not find any real advantages in the use of the GnRH antagonist for COS-IUI in terms of pregnancy rates.

Our group has been testing the use of the GnRH antagonist in COS-IUI (9), but from a different perspective. Our major concern was not premature luteinization, but follicular recruitment.

With this in mind, we did a preliminary study (9) and found that the inclusion of a GnRH antagonist in COS-IUI not only allowed the progression of all the recruited follicles to an ovulatory stage while avoiding unwanted LH release; it also achieved a significant increase in pregnancy rates (9). This result has been confirmed recently by other investigators (10, 11).

In any event, we hypothesized that the positive effect of the antagonist on IUI-cycle pregnancy rates is the result of a higher number of mature follicles (follicles >18 mm) and not of the GnRH antagonist itself, because COS-IUI pregnancy rates are known to be dependent on follicle number (9, 12–16).

However, because the results in the literature are contradictory in this regard, we undertook a new study with the aim of determining whether the association of multifollicular recruitment and GnRH antagonist in IUI cycles could significantly increase pregnancy rates.

Received January 16, 2007; revised and accepted March 12, 2007.
Reprint requests: Eleuterio R. Hernández, M.D., Ph.D., Clìnica de Medicina de la Reproducción y Ginecologìa "FivMadrid," Juan Álvarez de Mendizábal 74, 28008 Madrid, Spain (FAX: 34-915-610-700; E-mail: ehernandezm@fivmadrid.es).

0015-0282/08/$34.00
https://doi.org/10.1016/j.fertnstert.2007.03.035

MATERIALS AND METHODS

This study was a prospective, randomized clinical trial that was performed in 367 infertile couples who were recruited according to the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on human research and as approved by an ethics committee.

Inclusion Criteria

Women between 18 and 39 years of age, with regular menstrual cycles, primary or secondary infertility that had lasted for $\geq$12 months, a body mass index of between 19 and 28 kg/m^2, normal uterine cavity, bilateral tubal patency (assessed by hysterosalpingography and/or laparoscopy), normal PRL levels, and normal thyroid function were included in this study.

Only patients undergoing their first IUI cycle were included, and women with hormone values outside the reference range by day 3–4 of their menstrual period (FSH levels of >10 mIU/L), endometriosis, and/or polycystic ovarian syndrome were excluded.

Semen analysis was performed at least twice. Patients with a partner whose total motile spermatozoid swim-up count was <10 million/mL were excluded.

Controlled Ovarian Stimulation

Follicle-stimulating hormone group Before initiation, all patients (183 women) were examined by vaginal ultrasound (US) on the 3rd or 4th day of their menstrual period to ensure ovarian quiescence. Once a basal ovarian condition was established, a fixed dose of 75–150 U/d of recombinant FSH (rFSH, Gonal-F; Serono, Madrid, Spain or Puregon; Organon, Madrid, Spain) was given for 5 days to induce follicle recruitment.

At day 6 of ovarian stimulation, a vaginal US was performed to determine the number of follicles recruited, and a step-down protocol was initiated if the leading follicle was 13 mm in diameter. Controlled ovarian stimulation was monitored by US every 2 days.

When adequate ovarian response was observed and the follicular size of the leading follicle was between 18 and 20 mm in diameter, hCG (5,000 IU IM; hCG-Lepori; Farma-Lepori, Madrid, Spain), was administered.

Gonadotropin-releasing hormone antagonist group One hundred eighty-four patients were recruited for this group and subjected to the same COS protocol as described in the previous subsection, except that a daily dose of a GnRH antagonist (0.25 mg SC; Cetrotide, Serono or Orgalutran, Organon) was included once the leading follicle was $\geq$16 mm and was maintained until the day that hCG was given.

Cancellation of COS and IUI procedure If more than four follicles ($\geq$16–20 mm) were present by the day that hCG was administered, intrauterine insemination was canceled to reduce the risk of multiple pregnancy and to avoid ovarian hyperstimulation syndrome.

Another reason for canceling IUI was unexpected ovulation, which was assessed indirectly by the disappearance of the leading follicle and/or changes in the endometrial pattern (from a proliferative to a secretory phase) on US examination.

Semen Collection and Processing

Sperm was obtained from men in the clinical facilities by masturbation into a sterile jar after 2–3 days of abstinence. After liquefaction for approximately 20 to 30 minutes, the seminal fluid was diluted with 2 mL of Ham's F-10 medium (Gibco, Paisley, UK). The mixture was processed by centrifuge at 600 $\times$ g for 5–10 minutes, and the supernatant was discarded. Then, 0.3 mL of IVF Universal medium (Medicult, Jyllinge, Denmark) was added, and the mixture was incubated for 30–45 minutes at room temperature. After swim-up, the most active motile spermatozoa were isolated by aspiration, counted, and considered ready for IUI.

Procedure for IUI

Only one IUI was performed per cycle in both groups 36–38 hours after hCG, with a Gynetics catheter (Gynetics Medical Products, Hamont-Achel, Belgium). An insemination volume (0.3 mL) was delivered, and bed rest was maintained for 10 minutes after IUI.

Luteal-Phase Support

The same day after the IUI, natural micronized P (600 mg/d vaginally; Progeffik; Effik, Madrid, Spain or Utrogestan; Seid, Barcelona, Spain) was used to supplement the luteal phase. This regime was maintained until the 12th week of gestation or was discontinued if the pregnancy test was negative.

Pregnancy Assessment

Two weeks after insemination, an hCG assay (BioMerieux, France) was performed, and if positive, a transvaginal US was scheduled for 2 weeks later. Clinical pregnancy was defined as a positive hCG, together with the presence of a positive embryo heartbeat, as seen by US device equipped with a 5- to 7-Mhz endovaginal probe (Nemio; Toshiba Medical Systems, Tokyo, Japan).

Hormone Analyses

Serum levels of FSH, LH, E_2, and hCG were determined by using a commercial enzyme immunoassay kit (Vidas; Biomerieux, Spain). Blood samples were collected on the 3rd or 4th day of the cycle (E_2, LH, and FSH) and before hCG was given (E_2).

TABLE 1

Basal parameters.

Parameter	GnRH antagonist	Control
No. of patients	184	183
Age (y)	32.89 ± 2.5	32.05 ± 3.9
BMI (kg/m^2)	22.9 ± 1.7	23.4 ± 2.2
Basal FSH (mIU/L)	6.5 ± 2.3	6.6 ± 2.2
Basal LH (mIU/L)	5.3 ± 3.6	5.07 ± 3.3
Basal E_2 (pg/mL)	39.8 ± 22.1	48.7 ± 30.6

Note: Values are mean ± SD unless otherwise noted. *P* values were not significant when comparing the two groups on any parameter.

Gómez-Palomares. Ovarian stimulation and GnRH antagonist in IUI. Fertil Steril 2008.

Statistical Analysis

Patients were randomly assigned by using a computer-generated list that was compiled according to the order of their enrollment. The analysis was performed by using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD. Continuous variables were compared by using Student's *t* test. The χ^2 test and Fisher test were used to compare clinical outcome between the two groups. $P<.05$ was considered significant.

RESULTS

To evaluate the impact of the GnRH antagonist in IUI, this prospective randomized study subjected 367 patients to a protocol of COH with FSH+GnRH antagonist (n = 184 patients) or of rFSH alone (n = 183 patients).

As seen in Table 1, no significant differences in age (32.89 ± 2.5 y vs. 32.05 ± 3.9 y); BMI (22.9 ± 1.7 kg/m^2 vs. 23.4 ± 2.2 kg/m^2); and basal FSH (6.5 ± 2.3 mIU/mL vs. 6.6 ± 2.2 mIU/mL), LH (5.3 ± 3.6 mIU/mL vs. 5.07 ± 3.3 mIU/mL), or E_2 (39.8 ± 22.1 pg/mL vs. 48.7 ± 30.6 pg/mL) serum levels were seen between patients treated with rFSH+GnRH antagonist and those treated with rFSH alone, respectively.

Although the length of COS (7.8 ± 2.3 d vs. 6.9 ± 3.3 d) and the total amount of rFSH (698.1 ± 310 U vs. 629.5 ± 358 U) was slightly higher in the group of patients receiving the antagonist with respect to the group of patients receiving rFSH, none of these parameters reached significance (Table 2).

By the day that hCG was indicated, a nonsignificant decrease in E_2 serum levels (550 ± 172 pg/mL vs. 631 ± 205 pg/mL) and a significant increase in the number of recruited follicles (2.4 ± 1.3 vs. 1.3 ± 1.09; $P<.05$) had occurred in the group of patients receiving a GnRH antagonist (2 ± 1.2 ampoules) with respect to the control group. In this regard, 47% of the patients treated with FSH+GnRH antagonist and 45% of the patients treated with rFSH alone recruited one single follicle (Table 3).

Of the 367 cycles initiated, 21 (5.7%) were canceled (8 patients in the antagonist group and 13 in the control group); in 11 patients, this was a result of unexpected ovulation (2 patients in the antagonist group and 9 patients in the control group; $P<.05$), and in 10, it was done to avoid the risk of multiple pregnancy (6 patients in the antagonist group vs. 4 patients in the control group, a difference that was not statistically significant). None of the patients presented with any symptoms of ovarian hyperstimulation syndrome (Table 2).

In regard to pregnancy rates, 23% of the patients in whom COS-IUI was performed with rFSH+GnRH antagonist and 11% of the patients in whom COH-IUI was performed with FSH alone had a clinical pregnancy; this difference is statistically significant ($P<.05$). Nevertheless, if the IUI procedure was undertaken with only one follicle (of ≥18 mm), no

TABLE 2

Stimulation and cancellations.

Parameter	GnRH antagonist	Control	*P*
Ampoules (x)	2 ± 1.2	—	
FSH (total units)	698.1 ± 310	629.5 ± 358	NS
Days of COH	7.8 ± 2.3	6.9 ± 3.3	NS
No. of follicles ≥18 mm	2.4 ± 1.3	1.3 ± 1.09	<.05
E_2	550 ± 172	631 ± 205	NS
Swim-up (millions per mL)	25 ± 15.1	31.5 ± 20.8	NS
Cancellations by reason, n (%)	8 (4.3)	13 (7)	NS
Risk of multiple pregnancies	6 (3.2)	4 (2.1)	NS
Ovulation	2 (1.08)	9 (5)	<.05
Hyperstimulation syndrome	0	0	NS

Note: Values are mean ± SD. NS = not significant.

Gómez-Palomares. Ovarian stimulation and GnRH antagonist in IUI. Fertil Steril 2008.

TABLE 3

Pregnancy rates according to the number of mature follicles.

Parameter	GnRH antagonist	Control	*P*
Monofollicular cycles	47.1 (83/176)	45.8 (78/170)	NS
Pregnancies			
1 follicle of ≥18 mm	16.8 (14/83)	14.1 (13/92)	NS
≥2 follicles of ≥18 mm	33.3 (31/93)	8.9 (7/78)	<.05

Note: All data are % (n). NS = not significant.

Gómez-Palomares. Ovarian stimulation and GnRH antagonist in IUI. Fertil Steril 2008.

significant differences in pregnancy rates (16.8% vs. 14.1%) were found between the two groups (Table 3).

On US examination, 90% (38/42) of the pregnancies in the FSH+GnRH antagonist group and 73.6% (14/19) of the pregnancies in the control group were single gestations.

No significant differences were observed in the rate of twin pregnancies (4% vs. 15.7%) or miscarriage (9.5% vs. 10.5%) between the antagonist group and the control group (Table 4). There was one triplet gestation in the FSH+GnRH antagonist group and none in the FSH group.

DISCUSSION

In this study, we have demonstrated that the conjunction of multifollicular recruitment and GnRH antagonist in COS-IUI cycles may have a positive effect on pregnancy rates.

These results coincide with our work published elsewhere (9) and with work by other investigators (10, 11), and they appear to indicate that the inclusion of a GnRH antagonist in COS-IUI is a useful strategy, not just to control undesired luteinization (1, 6–11) but also to increase pregnancy rates (9–11).

Nevertheless, there are important studies in the literature that need to be taken into consideration, because they are well designed and have a significant number of patients, that did not find any differences in regard to pregnancy rates between patients treated or not with GnRH antagonist in COH-IUI cycles (1, 6, 8).

TABLE 4

Outcomes.

Parameter	GnRH antagonist	Control	*P*
Clinical pregnancy	23 (42/176)	11 (19/170)	<.05
Singles	90 (38/42)	73.6 (14/19)	NS
Twins	4 (2/42)	15.7 (3/19)	NS
Triplets	2.3 (1/42)	0 (0/19)	NS
Miscarriages	9.5 (4/42)	10.5 (2/19)	NS

Note: All data are % (n). NS = not significant.

Gómez-Palomares. Ovarian stimulation and GnRH antagonist in IUI. Fertil Steril 2008.

We believe that this discrepancy may be related to the number of preovulatory follicles that are recruited, and present, at the time that hCG is given. For example, if the number of recruited follicles in a COS-IUI cycle is reduced to a single follicle of size ≥18 mm, no differences in pregnancy rates between patients treated with rFSH+GnRH antagonist or patients with FSH alone (Table 3) are observed in our work. But on the contrary, if the hCG is administered, and IUI performed, when two to three preovulatory follicles (sized ≥18 mm) are present (which was the case in the group of patients who were treated with rFSH+GnRH antagonist), pregnancy rates significantly increased with respect to the group of patients that did not receive the antagonist (Table 3).

Similar results have been described in the literature (in regard to the number of preovulatory follicles and pregnancy rates) when IUI is undertaken in the presence of one single follicle (1, 6–8) or multifollicular recruitment (9–11) in the absence or presence of a GnRH antagonist and COH-IUI cycles.

In our opinion, the reason for the increase in the number of preovulatory follicles may be a permissive role of the antagonist. In other words, when the GnRH antagonist is introduced in the COS cycle, all the recruited follicles have the chance to grow to 20–22 mm without the risk of premature luteinization. Accordingly, the number of mature follicles and the possibility of pregnancy will increase (9, 12–16).

On the contrary, in the absence of GnRH antagonist, once the leading follicle is at the 18-mm boundary, and independently of the number and developmental status of the other recruited follicles, hCG is given. This standard procedure will reduce most of the COS-IUI cycles to a monofollicular category and will considerably decrease the chances of gestation, because pregnancy in IUI is related to the number of mature follicles that are present on the day hCG is indicated (9, 12–16).

We are fully aware that the increase in the number of mature preovulatory follicles may bring about a parallel increase in multiple-pregnancy rates and ovarian hyperstimulation

syndrome, because the link between these two parameters is well demonstrated in the literature (6, 17–19). Nevertheless, none of the patients included in this study, or in the one published elsewhere (9), developed any symptoms of ovarian hyperstimulation syndrome (Table 4), and with regard to the incidence of multiple pregnancies, >80% of the pregnancies were single, and <20%, twins.

It can be argued that by ignoring the cohort of follicles <14 mm in diameter, one leaves open the door to a multiple (high-order) pregnancy, because this connection is well established in the literature (17–19). However, our clinic's historical average for multiple gestations is <15% (twins), similar to what is described in the literature (17–19), and only one couple in the GnRH antagonist group had a triple gestation.

Another important aspect is to determine the minimum doses of GnRH antagonist that are necessary to obtain a benefit without compromising the economy of the patients and to avoid adverse effects (if any) in the final outcome of the COS-IUI cycle.

As seen in Table 2, the groups of patients treated with FSH+GnRH antagonist required only 2 ± 1.2 ampoules of the antagonist. That is to say, they have spent a minimum of 50 euros and a maximum of 150 euros. Considering that a COS-IUI cycle in Spain costs approximately 1,000 euros, the final increase is one of 10% with respect to the COS-IUI without antagonist. Similar conclusions were found by Allegra et al. (11).

In any event, some patients will find this increase worthwhile if the emotional and economical burden of cycle cancellation are taken into consideration. In this regard, in our study, a significant number of COS-IUI cycles were canceled in the group of patients who did not receive the GnRH antagonist, compared with in the group of patients who were treated with FSH+GnRH antagonist (Table 2). Significantly more COH-IUI cycles were canceled in the patients receiving FSH alone than in the group receiving the antagonist (Table 2).

Furthermore, to avoid increasing the cost of IUI unnecessarily, we did not determine levels of LH and E_2 (before the antagonist was given) or P (to evaluate premature luteinization) because, on the basis of our experience published elsewhere (9), the antagonist is now systematically introduced when the leading follicle is >16 mm in diameter.

In conclusion, our study demonstrates that treatment with the GnRH antagonist in COS-IUI cycles can improve their outcome, significantly raising the chances of achieving a pregnancy. This improvement is only evident in multifollicular cycles; thus, the use of GnRH antagonist in monofollicular COS-IUI cycles is not recommended, and only some specific reasons (i.e., weekends) will justify such use.

REFERENCES

1. Olivennes F, Fanchin R, Bouchard P, Taieb J, Frydman R. Triggering of ovulation by a gonadotropin-releasing hormone (GnRH) agonist in patients pretreated with a GnRH antagonist. Fertil Steril 1996;66:151–3.
2. Diedrich K, Diedrich C, Santos E, Zoll C, al-Hasani S, Reissmann T, et al. Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. Hum Reprod 1997;9:788–91.
3. European and Middle East Orgalutran Study Group. Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. Hum Reprod 2001;16:644–51.
4. Fluker M, Grifo J, Leader A, Levy M, Meldrum D, Muasher SJ, et al. North American Ganirelix Study Group. Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. Fertil Steril 2001;75:38–45.
5. Reissmann T, Felberbaum R, Diedrich K, Engel J, Comaru-Schally AM, Schally AV. Development and applications of luteinizing hormone-releasing hormone antagonists in the treatment of infertility: an overview. Hum Reprod 1995;10:1974–81.
6. Ragni G, Caliari I, Nicolosi AE, Arnoldi M, Somigliana E, Crosignani PG. Preventing high-order multiple pregnancies during controlled ovarian hyperstimulation and intrauterine insemination: 3 years' experience using low-dose recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. Fertil Steril 2006;85:619–24.
7. Matorras R, Ramon O, Exposito A, Corcostegui B, Ocerin I, Gonzalez-Lopera S, et al. Gn-RH antagonists in intrauterine insemination: the weekend-free protocol. J Assist Reprod Genet 2006;23:51–4.
8. Williams RS, Hillard JB, De Vane G, Yeko T, Kipersztok S, Rhoton-Vlasak A, et al. A randomized, multicenter study comparing the efficacy of recombinant FSH vs recombinant FSH with Ganirelix during super-ovulation/IUI therapy. Am J Obstet Gynecol 2004;191:648–51.
9. Gomez-Palomares JL, Julia B, Acevedo-Martin B, Martinez-Burgos M, Hernandez ER, Ricciarelli E. Timing ovulation for intrauterine insemination with a GnRH antagonist. Hum Reprod 2005;20:368–72.
10. Ragni G, Alagna F, Brigante C, Riccaboni A, Colombo M, Somigliana E, et al. GnRH antagonists and mild ovarian stimulation for intrauterine insemination: a randomized study comparing different gonadotrophin dosages. Hum Reprod 2004;19:54–8.
11. Allegra A, Marino A, Coffaro F, Scaglione P, Sammartano F, Rizza G, et al. GnRH antagonist-induced inhibition of the premature LH surge increases pregnancy rates in IUI-stimulated cycles. A prospective randomized trial. Hum Reprod 2007;22:101–8.
12. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 1999;14:698–703.
13. Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH, Pyrzak R. Effect of diagnosis, age, sperm quality, and number of preovulatory follicles on the outcome of multiple cycles of clomiphene citrate-intrauterine insemination. Fertil Steril 2002;78:1088–95.
14. Duran HE, Morshedi M, Kruger T, Oehninger S. Intrauterine insemination: a systematic review on determinants of success. Hum Reprod Update 2002;8:373–84.
15. Houmard BS, Juang MP, Soules MR, Fujimoto VY. Factors influencing pregnancy rates with a combined clomiphene citrate/gonadotropin protocol for non-assisted reproductive technology fertility treatment. Fertil Steril 2002;77:384–6.
16. Kaplan PF, Katz SL, Thompson AK, Freund RD. Cycle fecundity in controlled ovarian hyperstimulation and intrauterine insemination. Influence of the number of mature follicles at hCG administration. J Reprod Med 2002;47:35–9.
17. Tur R, Barri PN, Coroleu B, Buxaderas R, Martìnez F, Balasch J. Risk factors for high-order multiple implantation after ovarian stimulation with gonadotropins: evidence from a large series of 1878 consecutive pregnancies in a single centre. Hum Reprod 2001;16:2124–9.
18. Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH, Pyrzak R. Relationship of follicle numbers and estradiol levels to multiple implantation in 3,608 intrauterine insemination cycles. Fertil Steril 2001;75:69–78.
19. Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH, Pyrzak R. Risk factors for high-order multiple pregnancy and multiple birth after controlled ovarian hyperstimulation: results of 4,062 intrauterine insemination cycles. Fertil Steril 2005;83:671–83.

OBJETIVO 3

Demostrar que la maduración final de los oocitos con un agonista de la GnRH no compromete las tasas de implantación.

La maduración final con un agonista de la GnRH no compromete las tasas de implantación

Belén Acevedo, Jose Luis Gómez-Palomares, Elisabetta Ricciarelli, Eleuterio R. Hernández

Fertil Steril. 2006 Dec;86(6):1682-7

Las tasas de embarazo son significativamente menores cuando se utilizan los agonistas de la GnRH en lugar de la hCG para inducir la maduración final de los oocitos. Esto es debido a una luteolisis del cuerpo lúteo generada por el agonista que conlleva bajas concentraciones de LH, FSH, E2 e inhibina B (Griesinger et ál. 2005). Sin embargo la posibilidad de que exista un impacto directo del agonista en la calidad del oocito, en la viabilidad embrionaria y en el endometrio no se puede descartar. Por ello, el propósito de este estudio fue evaluar la capacidad de los embriones derivados de oocitos madurados con un agonista de la GnRH utilizando el modelo de la donación de oocitos. De esta forma el ovario es el único receptor del efecto agonista, liberando al factor endometrial al transferir los embriones a una mujer receptora.

El reclutamiento de las donantes se hizo siguiendo las recomendaciones de la Ley de Reproducción Asistida vigente en España en ese momento (*Ley* 35/1988, de 22 de noviembre, sobre Técnicas de *Reproducción Asistida*). 60 donantes fueron incluidas en el estudio. En todos los casos se administró en el ciclo previo al tratamiento un anticonceptivo hormonal oral. Las donantes fueron asignadas de forma aleatoria siguiendo un listado de números aleatorios generado por ordenador bien a un protocolo con agonista bien a un protocolo con hCG.

Tabla 1. Maduración final con un agonista de la GnRH. Parámetros basales, estimulación y reclutamiento y síndrome de hiperestimulación ovárica.

	Agonista	hCG	p
Número de donantes	30	30	
Edad (años)	21,56 ± 2,8	24,77 ± 1,4	ns
Niveles basales de FSH (mIU/mL) [a]	5,2 ± 1,2	4,4 ± 1,3	ns
Niveles basales de LH (mIU/mL) [a]	2,8 ± 0,3	2,3 ± 1,9	ns
Niveles basales de E2 (pg/mL) [a]	44,1 ± 9,3	32,5 ± 20,7	ns
Ampollas de antagonista [a]	4,46 ± 2,12	4,63 ± 1,41	ns
Dosis de FSH utilizada(UI) [a]	1612,5 ± 103	1605 ± 225	ns
Dosis de LH utilizada (UI) [a]	337,5 ± 174,2	441 ± 213	ns
E2 (pg/ml) día hCG/agonista [a]	2261,2 ± 195	1726,33 ± 354	ns
Tras 5 días de tratamiento			
Folículos >12 mm [a]	5,2 ± 1,2	3,1± 2,2	ns
Folículos <12 mm [a]	8,2 ± 1,9	9,2± 1,2	ns
Folículos día hCG/agonista [a]	11,56 ± 2,5	7,11± 3,4	ns
Días de estimulación [a]	10,4 ± 2,12	10,8± 1,89	
Duración de la fase lútea (días) [a]	4,16 ± 0,7	16,63 2,12	<0,05
Síndrome de hiperestimulación ovárica, n(%)	0/30 (0)	5/30 (16,6)	<0,05

[a] Media ± desviación estándar. Ns: no significativo.

Entre el tercero y el cuarto día del ciclo mensual se inició la estimulación ovárica con 150 unidades al día de FSH recombinante en todos los casos . El tratamiento se mantuvo durante cinco días. En el día sexto de la estimulación ovárica se introdujo el antagonista de la GnRH, (Ganirélix, MSD, 0,25 mg al día) que se mantuvo hasta el día de la maduración final del oocito. Coincidiendo con la introducción del antagonista se añadieron 75 UI al día de LH/HCG mantenidas hasta el final de la estimulación. En 30 donantes la maduración final oocitaria se realizó con hCG y en las 30 restantes se usó un agonista de la GnRH, triptorelina (Decapeptyl diario, Ipsen Pharma).

Tabla 2. Maduración final con un agonista de la GnRH. Calidad embrionaria.

	Agonista GnRH	hCG	p
Oocitos (n)	327	288	ns
Oocitos[a]	9.1 ± 4.01	10.3 ± 6.3	ns
Tasa Fecundación (%)	80	65	ns
Oocitos MI (%)	6	8	ns
Oocitos MII (%)	70	76	ns
Calidad Embronaria (%)			
Grado 1	35	50	ns
Grado 2	52	41	ns
Grad0 3	72	8	ns
Multinucleados	4	1	ns
Embriones con más de 8 blastómeras [a]	1,5 ± 1,2	1,9 ± 1,3	ns

[a] Media ± desviación estándar. Ns: no significativo.

Hubo un total de 60 receptoras de oocitos (rango de edad entre 34 y 47 años). 30 pacientes recibieron embriones procedentes de donantes en las que la maduración final se había hecho con un agonista de la GnRH y 30 embriones procedentes de donantes en las que se había utilizado hCG.

La preparación endometrial de las receptoras se realizó con estradiol (Progynova, Bayer) en todas las receptoras (Devroey y Pados, 1998). Un endometrio por encima de 8 mm se consideró apto para recibir embriones. Para evitar los embarazos múltiples no se transfirieron más de dos embriones.

La actividad de los cuerpos lúteos se objetivó indirectamente mediante la valoración de la duración de la fase lútea considerada como el tiempo transcurrido entre el día de la inducción de la ovulación y la aparición de la menstruación.

Pasados cinco días de estimulación en ambos grupos se observó un número similar de folículos mayores a 12 mm. También en ambos grupos se precisó una cantidad de FSH similar para reclutar un número equivalente de folículos maduros (Tabla 1).

No hubo diferencias ni en el número de oocitos recuperados ni en las tasas de fecundación ni en el porcentaje de oocitos

Tabla 3. Maduración final con un agonista de la GnRH. Resultados en las receptoras.

	Agonista GnRH	hCG	p
Receptoras (n)	30	30	
Oocitos donados [a]	5,9 ± 2,4	5,4 ± 3,1	ns
Embarazos/transferencia (%)	55	59	ns
Embarazos bioquímicos (%)	5	9	ns
Gestaciones clínicas (%)	84	90	ns
Tasa de implantación (%)	29	32	ns

ns: no significativo. [a] media ± desviación estándar.

maduros (MII) entre ambos grupos (Tabla 2). Es decir, el agonista de la GnRH puede provocar una liberación de LH suficiente como para promover la maduración de oocitos de la misma manera que la hCG .

En cuanto a la calidad embrionaria, evaluada morfológicamente, tampoco hubo diferencias significativas entre ambos grupos. Las tasas de embarazo así como las tasas de implantación en las receptoras fueron similares entre los dos grupos (Tabla 3). En cambio, sí hubo un aumento significativo en la duración de la fase lútea en las donantes en las que se había utilizado hCG en lugar del agonista. En cuanto a posibilidad desarrollar un síndrome de estimulación ovárica, ninguna de las donantes en las que se indujo la ovulación con el agonista desarrolló ninguna sintomatología, en cambio en el otro grupo hubo cuatro casos de estimulación moderada (Tabla 1).

A diferencia de otros trabajos, en este estudio se valoró el potencial implantatorio de los embriones en un endometrio, el de la mujer receptora, que no había sido influenciado por el agonista de la GnRH por lo que las tasas de embarazo, independientemente del origen de los oocitos, no se vieron afectadas (Tabla 3). Nuestro trabajo indicaba que la disminución en las tasas de implantación en

los ciclos en los que se usa un agonista de la GnRH para la ovulación puede deberse a una incapacidad del cuerpo lúteo para sintetizar los esteroides necesarios para el desarrollo de un endometrio en el que un embrión pueda implantar y desarrollarse durante los primeros estadíos del embarazo.

En este estudio se demostró por primera vez que los embriones derivados de oocitos madurados con agonista mantienen inalterado su capacidad de implantación y que la administración del agonista puede prevenir el síndrome de estimulación ovárica.

Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates

Belen Acevedo, M.D., Jose Luis Gomez-Palomares, M.D., Elisabetta Ricciarelli, M.D., and Eleuterio R. Hernández, M.D., Ph.D.

Clinica de Medicina de la Reproduccion y Ginecologia "FivMadrid," Madrid, Spain

Objective: To evaluate the implant capacity of embryos derived from oocytes matured with a bolus of GnRH agonist.
Design: Donors were randomly assigned to a protocol using either GnRH agonist or recombinant (r) hCG to trigger ovulation. Analysis of variance, Student *t* test, and Fisher exact test were used where appropriate.
Setting: Private clinical setting.
Patient(s): Young voluntary donors receiving GnRH agonist (n = 30) or rhCG (n = 30). Eighty-nine patients received oocytes.
Intervention(s): Controlled ovarian stimulation was carried out with GnRH antagonist and FSH/LH in a step-down protocol. Donors received a single bolus of GnRH agonist (0.2 mg) or rhCG (250 μg). The endometrial tissue of recipient patients was prepared with oral E_2 and P.
Main Outcome Measure(s): Pregnancy and implantation rates and ovarian hyperstimulation syndrome (OHSS) in an IVF donor program.
Result(s): No significant differences in the number of retrieved oocytes (327 vs. 288), MII oocytes (70% vs. 76%), fertilization (80% vs. 65%,), pregnancy/transfer (55% vs. 59%), and implantation rates (29% vs. 32%) were found between recipients whose embryos originated from donors in whom final oocyte maturation was triggered with GnRH agonist and those whose donors received hCG. Significant differences in luteal phase length (4.16 + 0.70 days vs. 13.63 + 2.12 days) and in OHSS (0/30 vs. 5/30) were seen between donors ovulated with the agonist and the donors in whom ovulation was triggered with hCG.
Conclusion(s): In controlled ovarian stimulation IVF donor cycles, GnRH agonists trigger ovulation and induce luteolysis but do not compromise embryo implantation capacity. (Fertil Steril® 2006;86:1682–7. ©2006 by American Society for Reproductive Medicine.)

Key Words: GnRH agonist, ovulation, oocyte donation, pregnancy, implantation

The introduction of GnRH antagonists in ovulation induction protocols has brought a renewed interest in the use of GnRH agonist to trigger ovulation (1–5).

There is an extensive body of evidence demonstrating that the GnRH agonists stimulate the release of physiologic levels of LH and FSH (1, 4–7). In addition, no differences in the number of MII oocytes, fertilization rates, or embryo development were seen in IVF-ET cycles in which ovulation was triggered with GnRH agonist instead of hCG (4, 5). Furthermore, triggering ovulation with the agonist would seem to be a useful strategy to prevent ovarian hyperstimulation syndrome (OHSS) (1, 7, 8).

Despite these observations, there have been significant decreases in IVF-ET cycle pregnancy rates in patients in whom final oocyte maturation was induced with GnRH agonists instead of the classic hCG (2, 5, 8). These disappointing results have been attributed to an irreversible luteolysis generated by the agonist and reflected in decreased plasma levels of P, E_2, and inhibin (2, 9–12).

Although the irreversible luteolysis is probably due to a profound negative steroid feedback that would suppress pituitary LH release (1, 12, 13), in our opinion another possibility for explaining the GnRH agonist–induced luteolysis would be some direct effect by the agonist on the luteinized granulosa cells. There is compelling evidence demonstrating that the human ovary is a target for GnRH reception and action (14). Also, the human ovary possesses an intrinsic GnRH axis composed of ligand and receptors (15–17) that is regulated during luteinization in an E_2-dependent manner (14). Additionally, in cultured luteinized granulosa cells, the GnRH agonist can mobilize Ca+ (18), activate mitogen protein kinases, increase c-fos mRNA expression (19), and decrease its steroidogenic capacity (19–21). Finally, and probably the most relevant for the fate of the corpus luteum, luteolysis would ultimately be due to the capacity of the GnRH agonist to initiate the intracellular cascade that drives the cell to programmed cell death or apoptosis (22, 23).

Received November 2, 2005; revised and accepted May 16, 2006.
Reprint requests: Eleuterio R. Hernández, Clinica de Medicina de la Reproduccion y Ginecologia, c/Juan Alvarez de Mendizabal 74, 28008-Madrid, Spain (FAX: 34 915610700; E-mail: ehernandezm@fivmadrid.es).

0015-0282/06/$32.00

doi:10.1016/j.fertnstert.2006.05.049

Although most of the published data at the present time seem to indicate that the GnRH agonist–induced decrease in implantation rate is mainly due to luteolysis, we believe that the agonist may have a direct impact on oocyte quality and hypothesize that this could influence the capacity of the embryo to be implanted.

Therefore, the purpose of this study was to evaluate the capacity of embryos derived from oocytes matured with a bolus of GnRH agonist to implant, using a protocol that can evaluate the impact of the agonist on the ovarian (folliculogenesis, oocyte maturation, and corpus luteum activity) compartment independently from its impact on the endometrial (implantation) compartment: the oocyte donor program (24, 25).

METHODS

Female donors were recruited following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on human research, and Institutional Review Board approval was obtained.

Briefly, the donors had to be older than 18 but not over 35, be healthy, and accept that oocyte donation is anonymous and altruistic. A consent form was provided and signed to be sure that they accepted and understood the IVF procedure. All donors had a normal menstrual cycle. Donors with polycystic ovaries, endometriosis, hydrosalpinges, and severe male factor (total number <5,000,000 spermatozoa) were excluded from this study to avoid any interference with the folliculogenesis process and pregnancy rates.

Sixty donors were subjected to a step-down protocol in which LH activity was suppressed with a GnRH antagonist as follows. To synchronize oocyte donation with patient uterine receptivity (and to avoid unexpected pregnancies), all donors received oral contraceptives (Etinilestradiol/Ciproterona Gineservice; Effik, Madrid, Spain) before controlled ovarian hyperstimulation was started.

Donors were randomly assigned with a computer-generated list to the GnRH agonist protocol or the recombinant (r) hCG protocol.

On the third or fourth day of their menstrual period, a fixed dose of 150 IU/day rFSH (Puregon; Organon, Barcelona, Spain) was given for 5 days to induce follicle recruitment. On day 6 of ovarian stimulation, 0.25 mg/day GnRH antagonist (Orgalutran; Organon) was injected SC and maintained until hCG or the GnRH agonist was given. Once the GnRH antagonist was initiated, 75 IU/day of LH (Menopur; Ferring, Madrid, Spain) was added and maintained until the GnRH antagonist was discontinued (24). Because Menopur is composed of highly purified (hp) FSH and LH (75 IU of each) the 75 IU hpFSH wase subtracted from the total rFSH (i.e., if 150 IU were the FSH dose, 75 IU would be supplied by hpFSH and 75 IU from the rFSH).

Subsequently, a step-down protocol was initiated and follicle development controlled by sequential ultrasound scans. In 30 donors, once the recruited follicles were >18 mm in diameter and if E_2 serum levels were <5,000 pg/mL, final oocyte maturation was obtained by SC injection of 250 μg/mL rhCG (Ovitrelle; Serono, Madrid, Spain), and oocyte retrieval was carried out under sedation and ultrasound guidance 32–34 hours later.

The other 30 donors were subjected to the same GnRH antagonist–FSH+LH step-down protocol, except final follicular maturation was obtained with a single bolus of 0.2 mg GnRH agonist triptoreline (Decapeptil; Ipsen, Barcelona, Spain), injected SC.

Intracytoplasmatic sperm Injection (ICSI) was routinely performed in all the fertilization procedures, as described elsewhere (26). Fertilization was evident when two pronuclei were observed. Embryos were cultured until the day of transfer (day 3) in IVF media (Vitrolife; Mölndalsvägen, Göteborg, Sweden) and graded by Veeck's (27) and Hsu et al.'s (28) criteria before transfer. In addition, it is our policy that any embryo with multinucleated blastomeres be excluded from transfer.

There were a total of 60 recipients (age range 34–47 years). Thirty patients received embryos originating from donors in whom final oocyte maturation and ovulation was triggered with GnRH agonist, and 30 received embryos originating from donors in whom final oocyte maturation and ovulation was induced with rhCG. Some donors gave oocytes to two recipients. Only one recipient was randomly included in the statistical analysis. The indications for oocyte donation were: ovarian failure (63%), four unsuccessful IVF cycles (32%), genetic (2%), and others (3%).

As previously described (22, 23), endometrial development was induced with E_2 (Progynova; Shering, Madrid, Spain) in all recipients. When the endometrial line evaluated by ultrasound scan was >8 mm, it was considered mature. To transform the endometrium to a secretory phase, 600 mg/day natural P (Progeffik; Effik) was given for at least 3 days before transfer. To avoid multiple pregnancies, two embryos were transfered to the uterine cavity under ultrasound monitoring. A pregnancy test (hCG; BioMerieux, Marcy-L'Etoile, France) was performed 15 days after embryo transfer. If positive, an ultrasound scan was scheduled for 2 weeks later to determine the number and status of implanted embryos. The concurrency of a positive hCG and embryo(s) with a positive heart beat (seen by ultrasound) was defined as a clinical pregnancy/transfer; otherwise it was considered to be a biochemical pregnancy/transfer. Implantation rates were calculated by dividing the number of sacs seen on ultrasound by the number of embryos transfered.

Hormonal control assays were determined with the use of a commercial enzyme immunoassay kit (Vidas; Biomerieux). Basal values of FSH, LH, and E_2 were routinely determined before the initiation of ovulation induction in any given cycle (day 3–4).

Analysis of variance (mean number of FSH ampules and oocytes retrieved), Student *t* test (implantation and pregnancy rates), and Fisher exact test were used where appropriate. $P<.05$ was considered significant.

The donors' corpus luteum activity was indirectly evaluated by luteal phase duration. For practical purposes, this was calculated considering the time lapse between the day ovulation was triggered by GnRH agonist or rHCG and the presence of bleeding (endometrial shading).

Ovarian hyperstimulation syndrome was classified following the criteria proposed by Rizk and Aboulghar (29). Briefly, five out of 30 donors of the hCG group developed mild OHSS: ovarian enlargement (5–7 cm), bloating, and minimal abdominal pain. None of them required hospitalization.

RESULTS

To determine if GnRH agonists affect embryo implantation rates, 60 selected donors were stimulated. As seen in Table 1, most of the donors had similar basal ovarian conditions as assessed by ultrasound and by serum gonadotropin level on the third or fourth day of their menstrual period. No significant differences in FSH (5.2 ± 1.2 mUI/mL vs. 4.4 ± 1.3 mUI/mL), LH (2.8 ± 0.3 mUI/mL vs 2.3 ± 1.9 mIU/mL), and E_2 (44.1 ± 9.3 pg/mL vs. 32.5 ± 20.7 pg/mL) levels were found between donors randomly assigned to ovulation with either GnRH agonist (30 donors) or rhCG (30 donors), respectively. Similarly, after 5 days of ovarian stimulation with a fixed dose of rFSH (150 IU/day), ultrasound revealed that a similar number of >12-mm (5.2 ± 2.8 vs. 3.1 ± 2.2) and <12-mm (8.2 ± 1.9 vs. 9.2 ± 1.2) follicles were recruited in both groups (Table 1).

Donor candidates for either GnRH agonist or rhCG to trigger ovulation had needed the same units of FSH (1,612.5 ± 103 vs. 1,605 ± 225, $P>.05$) and LH (337.5 ± 174.2 vs. 441 ± 213.7, $P>.05$) to recruit a similar number of mature follicles (11.56 ± 2.5 vs. 7.11 ± 3.4, $P>.05$) by the day the GnRH agonist or rHCG was administered. As a reflection of gonadotropin treatment, basal serum E_2 levels (41.5 ± 10.3 pg/mL vs. 30.7 ± 22.4 pg/mL) had significantly increased (2,261.2 ± 195 pg/mL vs. 1,726.33 ± 354 pg/mL) by the day that GnRH agonist or rHCG was administered (follicles >18 mm), respectively (Table 2).

No significant differences were seen in the total number of retrieved oocytes (327 vs. 288), fertilization rates (80% vs. 65%, $P>.05$), MI (6% vs 8%), and MII oocytes (70% vs. 76%, $P>.05$) between donors ovulated with GnRH agonist or with rhCG (Table 2).

TABLE 1

Triggering ovulation with GnRH agonist does not compromise embryo implantation rates.

	GnRH agonist	hCG	*P*
No. donors	30	30	
Age (yrs)	21.56 ± 2.8	24.77 ± 1.4	ns
Serum levels day 3			
FSH (mIU/mL)	5.2 ± 1.2	4.4 ± 1.3	ns
LH (mIU/mL)	2.8 ± 0.3	2.3 ± 1.9	ns
E_2 (pg/mL)	44.1 ± 9.3	32.5 ± 20.7	ns
GnRH antagonist (ampules)	4.46 ± 2.12	4.63 ± 1.41	ns
FSH (IU)	1,612.5 ± 103	1,605 ± 225	ns
LH (IU)	337.5 ± 174.2	441 ± 213	ns
E_2 (pg/mL) day hCG	2,261.2 ± 195	1,726.33 ± 354	ns
After day 5 of COH			
Follicles >12 mm	5.2 ± 1.2	3.1 ± 2.2	ns
Follicles <12 mm	8.2 ± 1.9	9.2 ± 1.2	ns
After day 5 of COH			
Follicles >12 mm	5.2 ± 1.2	3.1 ± 2.2	ns
Follicles <12 mm	8.2 ± 1.9	9.2 ± 1.2	ns
Folicles day hCG	11.56 ± 2.5	7.11 ± 3.4	ns
COH (days)	10.4 ± 2.12	10.8 ± 1.89	
Luteal phase (days)	4.16 ± 0.70	13.63 ± 2.12	<.05
OHSS rate (n)	(0/30)	(5/30)	
%	0	16.6	<.05

Note: ns = not significant; COH = controlled ovarian hyperstimulation.

Acevedo. GnRH agonists do not alter embryo implantation. Fertil Steril 2006.

TABLE 2

Triggering ovulation with GnRH agonist does not compromise embryo implantation rates.

	GnRH agonist	hCG	*P*
Oocytes (n)	327	288	ns
Oocytes (x)	9.1 ± 4.01	10.3 ± 6.3	ns
Fertilization rate (%)	80	65	ns
Oocytes MI (%)	6	8	ns
Oocytes MII (%)	70	76	ns
Embryo quality (%)			
Grade 1	35	50	ns
Grade 2	52	41	ns
Grade 3	72	8	ns
Multinucleated	4	1	ns
Embryos with >8 blastomeres (x)	1.5 ± 1.2	1.9 ± 1.3	ns

Note: ns = not significant; x = mean ± SD.

Acevedo. GnRH agonists do not alter embryo implantation. Fertil Steril 2006.

Regarding embryo quality, no significant differences in G1 (35% vs. 50%), G2 (52% vs. 41%), G3 (7% vs. 8%), and percentage of multinucleated (4% vs. 1%) embryos were observed between embryos derived from oocytes ovulated with either GnRH agonist or rhCG (Table 3). There was no difference in the cleavage status between both groups.

Interestingly, no differences in pregnancy/transfer (55% vs. 59%, $P>.05$), biochemical (5 vs. 9), clinical pregnancy (84% vs. 90%, $P>.05$), and implantation rates (29 vs. 32%, $P>.05$) were seen between the group of patients receiving embryos from donors in whom final oocyte maturation was induced with either GnRH agonist or rhCG (Table 3).

In contrast, as seen in Table 1, there was a significant difference in the length of luteal phase between the two groups. Donors that ovulated under the influence of the agonist menstruated during the first week (4.16 ± 0.70 days) after the GnRH agonist was applied, whereas the group of donors that ovulated under the influence of hCG menstruated 2 weeks after rhCG was applied (13.63 ± 2.12 days).

Regarding the possibility of developing OHSS, none of the donors (0 out of 30) in whom ovulation was triggered with the GnRH agonist had any symptoms of OHSS; however 4 out of 30 donors in whom ovulation was triggered with rHCG suffered mild OHSS (Table 1).

DISCUSSION

From our view point, it was important to evaluate embryo implantation because an increase in early pregnancy losses has been reported in IVF-ET cycles when ovulation is induced with GnRH agonist instead of hCG (1, 2, 4, 5). We completely agree that most of the losses could be related to irreversible GnRH agonist–dependent luteolysis (1, 8, 13), but it is still unknown (to the best of our knowledge) whether some of the early pregnancy losses could also be due (to some extent) to some alteration in the developmental program of the embryo.

Thus, to evaluate the implantation potential of embryos derived from cycles in which ovulation was induced with a GnRH agonist instead of with hCG, pregnancy rates were evaluated using oocyte donor cycles as a model (24, 25). This model was selected because the ovary would be the sole receptor of GnRH agonist action, thereby excluding the corpus luteum and the endometrium as factors in implantation failure. Furthermore, under our protocol, donors would receive the agonist treatment (affecting only the ovarian compartment) and the resulting embryos would be placed in an unexposed and unaffected endometrium (24, 25).

As expected, no differences were seen in the number of ampules of GnRH antagonist, units of gonadotropins (FSH/LH), E_2 serum levels, and number of mature follicles (>18 mm in diameter) between donors in whom final oocyte maturation was induced with GnRH agonist and those receiving hCG (Table 1).

The ability of GnRH agonist to induce ovulation in a physiologic manner has been demonstrated (1, 4, 6), and correspondingly no significant difference in the number of oocytes retrieved, percentage of MI and MII oocytes, and fertilization rates between donors in whom ovulation was triggered with GnRH agonist or hCG were seen (Table 2). These results coincide with previous reports and indicate that GnRH agonist can induce a physiologic release of LH that is sufficient to promote oocyte maturation to the same level as that generated by hCG (1, 4, 6, 7).

TABLE 3

Triggering ovulation with GnRH agonists does not compromise embryo implantation rates.

	GnRH agonist	hCG	*P*
No. recipients	30	30	
Oocytes donated	5.9 ± 2.4	5.4 ± 3.1	ns
Pregnancies/transfer (%)	55	59	ns
Biochemical pregnancy (%)	5	9	ns
Clinical pregnancy (%)	84	90	ns
Implantation rate (%)	29	32	ns

Note: ns = not significant.

Acevedo. GnRH agonists do not alter embryo implantation. Fertil Steril 2006.

In contrast to the undesirable results obtained in IVF-ET cycles in which ovulation was triggered with the GnRH agonist, which were manifested as irreversible luteolysis and very poor pregnancy rates (1, 2, 5), no significant differences in pregnancy rates or any of the parameters related with implantation were seen in any of the patients who received embryos originating from oocytes whose donors had received GnRH agonist compared with those receiving hCG for final oocyte maturation (Table 3). Thus, these results practically exclude the embryo as a factor in considering the etiology of the decreases in early implantation seen in IVF-ET and leave the luteinized granulosa cells of the ovary as the main target for GnRH agonist reception and action.

Our results can be seen as expected, because no differences in oocyte morphology or embryo quality was evident not only in our study but also in similar reports in the literature (1, 2, 4, 5). Nevertheless, we have already shown that oocyte and embryo quality evaluated by classic microscopic parameters does not necessarily correlate with pregnancy rates (24, 25).

Regarding the fate of the corpus luteum, it has been shown that GnRH agonist is able to initiate the intracellular signaling cascade that induces apoptosis in the luteinized granulosa (22, 23). In consequence, it could be appropriate to speculate that under GnRH agonist stimulation the corpus luteum may not attain the capacity to synthesize the steroids required to develop an endometrium that could sustain the early stages of implantation.

In this connection, the length of the donor luteal phase was seriously altered in our study, because endometrial shade (bleeding) occurs 1 week post-agonist in the group of donors in whom ovulation was triggered with the GnRH agonist, whereas in the group of donors ovulated with hCG the luteal phase lasted for 2 weeks (Table 1). Our results parallel those described in IVF-ET (4, 8, 9, 12, 13) and indicate that corpus luteum performance is seriously affected when ovulation is induced with GnRH agonist.

Although luteolysis induced by GnRH agonist does not exclude the possibility of OHSS (9, 30, 31), in our study none of the donors that ovulated with the GnRH agonist suffered from OHSS; on the other hand, four cases of mild OHSS were detected in the group of donors receiving rhCG (Table 1). Therefore, in our opinion, the use of GnRH agonist instead of hCG to induce final oocyte maturation can be a good alternative to avoid OHSS in assisted reproduction treatments (1, 4, 6, 31).

In conclusion, we have demonstrated (for the first time) that in embryos derived from oocytes matured with GnRH agonist, embryo implantation is unaltered and the reported decrease in pregnancy rates seems to be associated with luteolysis. From our view point, this was important to clarify because when GnRH agonist is used to prevent OHSS, whether in GnRH antagonist/IVF-donor cycles or GnRH antagonist/IVF-ET cycles (in which most of the embryos will be cryo-preserved and transfered in a subsequent cycle), pregnancy and implantation rates will rely on embryo integrity; our results support that integrity.

Acknowledgment: We would like to thank Dr. Antonio Pareja for his valuable assistance with the statistical analysis.

REFERENCES

1. Kol SK. Luteolysis induced by a gonadotropin-releaseg hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fetil Steril 2004;81:1–5.
2. Humaidan P, Ejdrup Bredkjaer H, Bungum L, Bungum M, Grondahl ML, Westergaard L, Yding Andersen C. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum Reprod 2005;20:1213–20.
3. Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report: short communication. Hum Reprod 2000;15:1965–8.
4. Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J, van Hooren HG. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist hyperstimulation for in vitro fertilization. J Clin Endocrinol Metab 2002;87:709–15.
5. Kolibianakis EM, Schultze-Mosgau A, Schroder A, van Steirteghem A, Devroey P, Diedrich K Griesinger G. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocytes maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005;20:2887–92.
6. Tay CC. Use of gonadotropin-releasing hormone agonist to trigger ovulation. Hum Fertil (Camb.) 2002;5:G35–7.
7. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L, Brandes JM. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril 1991;56:213–20.
8. Balach J, Tur R, Creus, Buxaredas R, Fabregues F, Ballesca JL, et al. Triggering of ovulation by a gonadotropin releasing hormone agonist in gonadotropin-stimulated cycles for prevention of ovarian hyperstimulation syndrome and multiple pregnancy. Gynecol Endocrinol 1994:7–12.
9. Gerris J, De Vits A, Joostens M, Van Royen E. Triggering of ovulation in human menopausal gonadotrophins in stimulated cycles: comparison between intravenously administration of gonadotrophins-releasing hormone (100 and 500 micrograms) and GnRH agonist (buserelin, 500 micrograms) and human chorionic gonadotrophin (10000 IU). Hum Reprod 1995;10:56–62.
10. Segal S, Casper RF. Gonadotropin-releasing hormone agonist versus human chorionic gonadotropin for triggering follicular maturation in vitro fertilization. Fertil Steril 1992;57:1254–8.
11. Nevo O, Eldar-Geva T, Kol S, Itskovitz-Eldor J. Lower levels of inhibin A and pro-alphaC during the luteal phase after triggering oocytes maturation with a gonadotropin-releasing hormone agonist versus human chorionic gonadotropin. Fertil Steril 2003;79:1123–8.
12. Lanzone A, Fulghesu AM, Villa P, Guida C, Guido M, Nicoletti MC, et al. Gonadotropin-releasing hormone agonist versus human chorionic gonadotropin as a trigger of ovulation in polycystic ovarian disease gonadoptropin hyperstimulated cycles. Fertil Steril 1994;62:35–41.
13. Bechers NG, Macklon NS, Eijkemans MJ, Ludwig M, Felberbaum RE, Diedrich K, et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in vitro fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. J Clin Endocrinol Metab 2003;88:4186–92.

14. Nathwani PS, Kang SK, Cheng KW, Choi KC, Leung PC. Regulation of gonadotropin-releasing hormone and its receptor gene expression by 17beta-estradiol in cultured human granulosa-luteal cells. Endocrinology 2000;141:1754–63.
15. Kang SK, Tai CJ, Nathwai PS, Choi KC, Leung PC. Stimulation of mitogen-activated protein kinase by gonadotrophin-releasing hormone in human granulosa-luteal cells. Endocrinology 2001;142:671–679.
16. Brus L, Lambalk CB, de Koning J, Helder MN, Janssens RM, Schoemaker J. Specific gonadotrophin-releasing hormone analogue binding predominantly in human luteinized follicular aspirates and not in human pre-ovulatory follicles. Hum Reprod 1997;12:769–73.
17. Minaretzis D, Jakubowski M, Mortola JF, Pavlou SN. Gonadotrophin-releasing hormone receptor gene expression in human ovary and granulosa-lutein cells. J Clin Endocrinol Metab 1995;80:430–4.
18. Hori H, Uemura T, Minaguchi H. Effects of GnRH on protein kinase C activity, Ca2+ mobilization and steroidogenesis of human granulosa cells. Endocr J 1998;45:175–82.
19. Kang SK, Tai CJ, Nathwani PS, Leung PC. Differential regulation of two forms of gonadotrophin-releasing hormone messenger ribonucleic acid in human granulosa-luteal cells. Endocrinology 2001;142:182–92.
20. Dor J, Bider D, Shulman A, Levron JL, Shine S, Mashiach S, Rabinovici J. Effects of gonadotrophin-releasing hormone agonists on human ovarian steroid sex ration in vivo and in vitro—results of a prospective, randomized in-vitro fertilization study. Hum Reprod 2000;15:1225–30.
21. Gaetje R. Influence of gonadotrophin releasing hormone (GnRH) and a GnRH-agonist on granulosa cell steroidogenesis. Clin Exp Obstet Gynecol 1994;21:164–9.
22. Yano T, Yano N, Matsumi H, Morita Y, Tsutsumi O, Schally AV, Taketani Y. Effect of luteinizing hormone–releasing hormone analogs on the rat ovarian follicle development. Horm Res 1997;48:35–41.
23. Takekida S, Deguchi J, Samoto T, Matsuo H, Maruo T. Comparative analysis of the effects of gonadotropin-releasing hormone agonist on the proliferative activity, apoptosis and steroidogenesis in cultured porcine granulosa cells at varying stages of follicular growth. Endocrine 2000;12:61–7.
24. Ricciarelli E, Sánchez M, Martinez M, Andres L, Cuadro J, Hernández ER. Impact of the GnRH antagonist in oocyte donation cycles. Fertil Steril 2003;79:1461–3.
25. Acevedo B, Sanchez M, Gomez JL, Cuadros J, Ricciarelli E, Hernández ER. Luteinizing hormone supplementation increases pregnancy rates in gonadotropin-releasing hormone antagonist donor cycles. 2004;82:343–7.
26. Palermo GP, Joris H, Devroey P, Van Steirteghem AC. Pregnancy after intracytoplasmatic injection of a single spermatozoon into an oocyte. Lancet 1992;340:17–18.
27. Veeck LL. Atlas of human gametes and conceptuses. New York: Parthenon, 1999.
28. Hsu MI, Mayer J, Aronshon M, Lanzendorf S, Muasher S, Kolm P, Oehninger S. Embryo implantation in in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. Fertil Steril 1999;72:679–85.
29. Rizk B, Aboulghar MA. Classification, pathophysiology and management of ovarian hyperstimulation syndrome. In: Brinsden P, ed. In vitro fertilization and assisted reproduction. New York, London: Parthenon, 1999;131–55.
30. Van der Meer S, Gerris J, Joostens M, Tas B. Tiggering of ovulation using a gonadotropin-releasing hormone agonist does not prevent ovarian hyperstimulation syndrome. Human Reprod 1993;8:1628–31.
31. Ragni G, Vegetti W, Riccaboni A, Engl B, Brigante C, Crosignani PG. Comparison of GnRH agonists and antagonists in assisted reproduction cycles of patients at high risk of ovarian hyperstimulation syndrome. Hum Reprod 2005;20:2421–5.

OBJETIVO 4

Aseverar que en los ciclos de donación de oocitos, no ha lugar la cancelación, el "coasting" o el síndrome de hiperestimulación ovárica si se usan los antagonistas de la GnRH para inducir la ovulación.

No ha lugar para la cancelación, el coasting o el síndrome de hiperestimulación ovárica en los ciclos de donación de oocitos.

Eleuterio R. Hernández, José Luis Gómez-Palomares, Elisabetta Ricciarelli

Fertil Steril. 2009 Apr;91(4):1358-61

En el estudio anterior, nos llamó la atención que ninguna de las donantes desarrollara un SHO independientemente de los valores plasmáticos de estradiol. Para confirmar este hallazgo hicimos un análisis retrospectivo de 429 ciclos de FIV con donación de ovocitos, en el que se compararon los ciclos de ovodonación en el que la inducción de la ovulación se indujo bien con hCG o con agonista de la GnRH.

En 119 donantes (grupo hCG) se indujo la foliculogénesis con FSH (urinaria y recombinate), la supresión hipofisaria se llevo acabo con agonista o bien con un antagonista de la GnRH y la ovulación siempre con hCG.

En las donantes del grupo agonista (176) la estimulación fue similar excepto que la supresión hipofisaria se hizo siempre con un antagonista de la GnRH y la ovulación inducida con un agonista.

Un total de 694 mujeres recibieron oocitos procedentes de las donaciones (algunas donantes dieron oocitos a dos receptoras). 340 pacientes recibieron embriones originados por donantes en las cuales la maduración se había realizado con hCG y 380 embriones originados por donantes en las que se había utilizado el agonista de la GnRH como inductor final de la ovulación.

Las donantes del grupo agonista reclutaron un número mayor de folículos, la estimulación fue más corta y la

concentración en sangre de estradiol fue significativamente más alta (Tabla 1).

Tabla 1. No ha lugar para la cancelación, el coasting o el síndrome de hiperestimulación ovárica en los ciclos de donación de oocitos. Estimulación y recuperación oocitaria.

	Grupo hCG	Grupo Agonista	
Número de donantes	119	176	
Número de ciclos	175	254	
Edad (años)[a]	24,25 ± 4,4	23,67 ± 3,5	0,15
Niveles de FSH en D3, mIU/mL[a]	5,4 ± 1,5	6,1 ± 1,4	0,14
Niveles de LH en D3, mIU/mL[a]	3,1 ± 1,2	3,2 ± 2,8	0,65
Niveles de estradiol en D3, pg/mL[a]	32,03 ± 12,2	29,6 ± 8,8	0.49
Índice de masa corporal kg/m^2[a]	21,3 ± 3,9	19,6 ± 4,2	0,77
Unidades de FSH[a]	1345,9 ± 386	1652 ± 388	<0,001
Folículos >18 mm[a]	9,9 ± 4	15,1 ± 6,1	<0,001
Estradiol final[a]	1653 ± 1038	2987 ± 1879	<0,001
Días de estimulación[a]	10.4 ± 2	9 ± 1.5	<0,001
Oocitos[a]	1442 (8,7 ± 5,2)	2688 (13,2 ± 7,2)	<0,001
Oocitos maduros, %	79.00	72.00	0,32
Embriones de Grado 1, %	55	42	0,08
Embriones de Grado 2, %	40	51	15

[a]Media ± desviación estándar.

En el grupo de donantes que recibieron hCG (10.000 UI), cinco ciclos fueron cancelados por riesgo de HSO y en dos fue preciso realizar un "coasting" (suspender la estimulación hasta que se produce un descenso adecuado en los niveles de E2) con el fin de bajar los niveles plasmáticos de estradiol (<4000 pg/ml) y poder realizar la punción folicular. Por el contrario, y a pesar de los altos niveles de estradiol, ninguno de los ciclos de ovodonacion del grupo agonista tuvo que ser cancelado o tuvo que realizarse "coasting" (Tabla 2).

En relación al síndrome de hiperestimulación ovárica, de las 119 donantes del grupo hCG , 10 de ellas (3,2%) desarrolló un cuadro de HSO de grado moderado (2,2%) o severo (1%). Sin embargo ninguna de las 176 donantes que recibieron el agonista sufrió SHO alguno. Debido a la luteolisis que genera el agonista de

Tabla 2. No ha lugar para la cancelación, el coasting o el síndrome de hiperestimulación ovárica en los ciclos de donación de oocitos. Fase lútea y complicaciones.

	Grupo hCG	Grupo Agonista	
Duración de la fase lútea (días)[a]	12.84 ± 3. 2	3.9 ± 1.7	0.001
Coasting (n) (%)	2 (0.6)	0	0.52
Cancelaciones por riesgo de SHO (n) (%)	5 (1.6)	0	0.16
SHO total (n) (%)	10 (3.2)	0	0.007
SHO moderado	7 (2.2)	0	0.04
SHO severo	3 (1)	0	

[a]Media ± desviación estándar.

la GnRH, la duración de la fase lútea fue significativamente más corta en este grupo, respecto del grupo hCG (Tabla 2).

Las tasas de fecundación, porcentaje de oocitos en metafase dos y la calidad embrionaria fueron similares en ambos grupos. Un total de 694 pacientes compartieron los 4.130 oocitos. No hubo diferencias significativas en las tasas de embarazo clínico, implantación o porcentaje de embarazos gemelares en las receptoras de embriones procedentes del grupo hCG o agonista (Tabla 3).

Hasta la disponibilidad de los antagonistas de la GnRH no

Tabla 3. No ha lugar para la cancelación, el coasting o el síndrome de hiperestimulación ovárica en los ciclos de donación de oocitos. Resultados en las receptoras.

	Grupo hCG	Grupo Agonista	
Número de ciclos de receptoras	314.00	380.00	
Edad (años)[a]	38,9 ± 5,2	38,2 ± 5	0,22
Oocitos recibidos[a]	5,1 ± 1,8	6,5 ± 2,2	1.00
Embarazos/trasnferencias, %	52.00	51.00	0,99
Tasa de implantación, %	33.00	31.00	0,87
Gestaciones gemelares, %	16.00	19,4	0,58

[a]Media ± desviación estándar.

existía la posibilidad de madurar los folículos con un agonista de la GnRH por lo que cualquier mujer joven que de forma altruista se sometía a una donación de oocitos corría un riesgo para su salud por la posibilidad de desarrollar un SHO. El hecho de que ninguna

de las donantes del grupo agonista desarrollara un SHO, convierte en obligatorio que la estimulación ovárica en la ovodonación se realice en el contexto de un protocolo con antagonista y agonista.

No room for cancellation, coasting, or ovarian hyperstimulation syndrome in oocyte donation cycles

We retrospectively studied 429 IVF donor cycles in which ovulation was triggered with either hCG (175 cycles) or GnRH agonist (254 cycles). Of the donors in whom ovulation was triggered with hCG, 3.2% developed symptoms of moderate (2.2%) or severe (1%) ovarian hyperstimulation syndrome, while none of the IVF donor cycles that were triggered with the GnRH agonist presented ovarian hyperstimulation syndrome, needed coasting, or were cancelled. (Fertil Steril® 2009;91:1358–61. ©2009 by American Society for Reproductive Medicine.)

Oocyte donation is a well-known and established alternative for patients with ovarian failure, repeated IVF failure, diminished ovarian reserve, compromised oocyte quality, and physiological menopause and for single women. These circumstances create a strong demand for oocytes. Meanwhile, worldwide, the number of recipient-patients is steadily increasing, and the number of potential donors is falling short of the demand. Thus donors sometimes receive high FSH doses to induce folliculogenesis and obtain a fairly large number of oocytes per donor.

Most of the time, this strategy does not present problems for the well-being of the donors; however, on some occasions there will be an overresponse that may result in cycle cancellation, coasting, or ovarian hyperstimulation syndrome (OHSS) (1–4). All these inconveniences, although well-known and partially tolerated by the IVF patient, are not well understood and are taken badly by the donor, ultimately endangering the enrollment of potential new donors. On the other hand, oocyte recipients, who have already invested important resources (5) in the donation procedure (selection, medication, traveling, etc), can be disappointed if the donor's cycle needs to be coasted or the whole procedure cancelled because of a possibility of donor hyperstimulation.

In our opinion, these undesirable situations can be controlled and avoided and implantation rates can still be maintained if final oocyte maturation is induced with a GnRH agonist instead of hCG in the oocyte donation cycles (6, 7), contrary to what has been described in IVF transfer using agonist-induced ovulation (8–10).

To this end, a retrospective data analysis of 429 IVF donor cycles (performed between January 2003 and December 2006) was done to compare adverse complications (cancellation, coasting, and OHSS) in two periods. In the first period, ovulation was induced by hCG, and in the second it was induced by GnRH agonist. Thus, donors received either hCG or the agonist depending on when they donated, before or after the GnRH antagonist became available. Two hundred ninety-five voluntary female donors, recruited following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, were subjected to controlled ovarian hyperstimulation (COH). Our Institutional Review Board approved this analysis. In 119 donors (hCG group), folliculogenesis was induced with recombinant FSH (Gonal F, Serono, Madrid, Spain; or Puregon, Organon, Barcelona, Spain) or purified urinary gonadotropin (Menopur, Ferring, Madrid, Spain). LH suppression was achieved with GnRH agonist (Decapeptil 3.75 mg, IpsenPharma, Barcelona, Spain) or GnRH antagonist (Cetrotide, Serono; Orgalutran, Organon). Ovulation was triggered with urinary hCG (hCG-Lepori, Lepori, Barcelona, Spain) or recombinant hCG (Ovitrelle, Serono), as described elsewhere (6, 11). In the GnRH agonist group, 176 donors were subjected to COH as described above, except that LH was always suppressed with GnRH antagonist and ovulation triggered with a single bolus of 0.2 mg of GnRH agonist (Decapeptyl, IpsenPharma), as described elsewhere (6).

Intracytoplasmatic sperm injection was routinely performed in all the fertilization procedures. Embryos were cultured until the day of transfer (day 3), graded before transfer (6, 11), and, to avoid high-order multiple pregnancies, two embryos were transferred. There were a total 694 donations (some donors gave oocytes to two recipients). Three hundred fourteen patients received embryos originating from donors in whom ovulation was triggered with hCG, and 380 received embryos originating from donors in whom ovulation was triggered with the agonist. Endometrial preparation was done as described elsewhere (6, 11). A pregnancy test was performed 15 days after ET. The concurrency of a positive hCG and embryo(s) with a positive heartbeat (seen by ultrasound) was defined as a clinical pregnancy; otherwise it was considered a biochemical pregnancy. Implantation rates were calculated by dividing the

Received October 17, 2007; revised and accepted March 28, 2008; published online June 13, 2008.
E.R.H has nothing to disclose. J.L.G.-P. has nothing to disclose. E.R. has nothing to disclose.
Reprint requests: Eleuterio R. Hernández, M.D., Ph.D., Clínica de Medicina de la Reproducción y Ginecología "FivMadrid," c/Juan Álvarez de Mendizábal 74, 28008-Madrid, Spain (FAX: 34-915610700; E-mail: ehernandezm@fivmadrid.es).

0015-0282/09/$36.00

doi:10.1016/j.fertnstert.2008.03.077

number of sacs seen on ultrasound by the number of transferred embryos.

Data for this retrospective study were collected from our medical record database. For descriptive statistics, we used means ± SD. Student's *t*-test, Fisher's exact test, and the χ^2-test were used where appropriate. $P<.05$ was considered statistically significant. Coasting was performed and OHSS was classified following the criteria proposed by the Practice Committee of the American Society for Reproductive Medicine (12).

As seen in Table 1, no significant differences in age (24.25 ± 4.4 vs. 23.67 ± 3.5 years), body mass index (21.3 ± 3.9 vs. 19.6 ± 4.2 kg/m^2), FSH (5.4 ± 1.5 vs. 6.1 ± 1.4 mIU/mL), LH (3.1 ± 1.2 vs. 3.2 ± 1.8 mIU/mL), or E_2 (32.03 ± 12.2 vs. 29.6 ± 8.8 pg/mL) serum basal levels on the third or fourth day of their menstrual period were found between donors assigned to ovulate with either hCG or GnRH agonist. Donors in whom ovulation was triggered with a GnRH agonist received a significantly greater amount of FSH (1652.74 ± 388.9 vs. 1345.9 ± 386 IU) and recruited significantly more mature follicles (15.1 ± 6.1 vs. 9.9 ± 4.05) and oocytes (13.2 ± 7.2 vs. 8.7 ± 5.2 oocytes/donor) with a shorter COH (9 ± 1.5 vs. 10.4 ± 2.08 days) than the donors ovulated with hCG.

On the day the agonist or hCG was indicated, serum E_2 levels were significantly higher in the agonist group (2987 ± 1879.77 pg/mL) than in the hCG group (1653.57 ± 1038.8 pg/mL). Five cycles were cancelled, and two needed coasting in the hCG group. However, despite the high E_2 levels, none of the cycles in the GnRH agonist group were cancelled or coasted.

TABLE 1

Demography, cycle characteristics, embryo quality, and recipient outcomes in oocyte donation cycles ovulated with either hCG or GnRH agonist.

	hCG	GnRH agonist	*P*
No. of donors	119	176	
No. of cycles	175	254	
Age, years	24.25 ± 4.4	23.67 ± 3.5	.15
Day 3 serum levels			
FSH, mIU/mL	5.4 ± 1.5	6.1 ± 1.4	.14
LH, mIU/mL	3.1 ± 1.2	3.2 ± 1.8	.65
E_2, pg/mL	32.03 ± 12.2	29.6 ± 8.8	.49
Body mass index, kg/m^2	21.3 ± 3.9	19.6 ± 4.2	.77
FSH, IU	1345.9 ± 386	1652.74 ± 388.9	<.001
Follicles >18 mm on day of hCG administration	9.9 ± 4.05	15.1 ± 6.1	<.001
E_2 on day of hCG administration, pg/mL	1653.57 ± 1038.8	2987 ± 1879.77	<.001
COH, days	10.4 ± 2.08	9 ± 1.5	<.001
Oocytes	1442 (8.7 ± 5.2)	2688 (13.2 ± 7.2)	<.001
Fertilization rate, %	80	75	.49
Metaphase II oocytes,%	79	72	.32
Grade 1 embryos, %	55	42	.08
Grade 2 embryos, %	40	51	.15
Luteal phase length, days	12.84 ± 3. 2	3.9 ± 1.7	.001
Coasting (%)	2 (0.6)	0	.52
Cancel risk OHSS (%)	5 (1.6)	0	.16
OHSS (%)	10 (3.2)	0	.007
Moderate (%)	7 (2.2)	0	.04
Severe (%)	3 (1)	0	.28
No. of cycles of recipients	314	380	
Age, years	38.9 ± 5.2	38.2 ± 5	.22
Oocytes donated	5.1 ± 1.8	6.5 ± 2.2	.001
Pregnancies/transfer, %	52	51	.99
Implantation rate, %	33	31	.87
Twins, %	16	19.4	.58

Note: Data are mean ± SD unless otherwise specified.

Hernández. Safer protocol for oocyte donation cycles. Fertil Steril 2009.

Regarding OHSS, 3.2% of the 119 donors in whom final oocyte maturation was induced with hCG developed symptoms of moderate (2.2%) or severe (1%) OHSS. However, none of the 176 donors receiving GnRH agonist to trigger ovulation developed OHSS. Furthermore, the length of the luteal phase (3.9 ± 1.7 vs. 12.84 ± 3.2 days) was significantly shorter in donors ovulated with GnRH agonist than in those receiving hCG.

Fertilization rates (80 % vs. 75%), metaphase II oocytes (79% vs. 72%), and embryo quality (grade 1, 55% vs. 42%; grade 2, 40% vs. 51%) were not significantly different between donors ovulated with hCG or GnRH agonist, respectively.

A total of 694 patients shared the 4130 oocytes retrieved. No significant differences in clinical pregnancy/transfer (52% vs. 51%), implantation rates (33% vs. 31%), or twin pregnancies (16% vs. 19.4%) were noted between the groups of patients receiving embryos from oocytes originating in donors ovulated with either hCG or the GnRH agonist, respectively.

To the best of our knowledge, this retrospective study evaluates the largest number of GnRH agonist–induced oocyte donation cycles yet published. None of the donors who ovulated with a GnRH agonist instead of hCG needed coasting, were cancelled, or developed any type of OHSS complication. From the strategic viewpoint, this is very important because sometimes young healthy donors with a normal endocrine profile are subjected to COH with more FSH units than IVF patients in the same age range to recruit sufficient follicles and oocytes to ensure a minimum of six to eight oocytes/patient and, if possible, to service two recipients. As a consequence of the large FSH dose, some of the donors may develop OHSS, particularly if ovulation is triggered with hCG (1–4, 12).

In this regard, since we began our oocyte donation program, three of our donors (1%) in the hCG group had serious complications related to OHSS (two required hospitalization), and this is in the range described with IVF-ET (1, 4). A donor who develops OHSS, like many infertile patients who have suffered the unpleasant and dangerous experience of OHSS, will never want to repeat the experience and probably never recommend donating to a friend (personal experience). Furthermore, if we also consider the number of donors who frequently develop hCG-related ovarian enlargement, pain, and/or discomfort that can require at least 2 weeks to wear off, the idea of donation is not very attractive. On the other hand, coasting is a partial solution that will not guarantee that OHSS will not develop or that the cycle will ultimately not be cancelled (1). Thus, coasting will alter the life plans of donors and patients, increase daily clinic visits, lead to more E_2 determinations, and add to the final cost.

A completely different picture was seen in the group of donors ovulated with GnRH agonist, not only in respect to complications (coasting, cancellation, OHSS) but also in respect to their feelings regarding the donation procedure. As described above, the total amount of FSH, number of follicles recruited, and oocytes retrieved per cycle were significantly increased in the GnRH agonist group compared with the hCG group. Nevertheless, no significant differences in the percentage of metaphase II oocytes, fertilization rates, pregnancy rates, or any of the other parameters related to implantation were evident between donors in whom ovulation was triggered with either GnRH agonist or hCG.

Altogether, these results reconfirm that GnRH agonist can induce a physiological release of LH by gonadotropin that is enough to promote oocyte maturation similar to the one obtained with hCG (13–15). What is more, contrary to the undesirable results obtained in IVF-ET, in which the GnRH agonist results in an irreversible luteolysis (13) and poor pregnancy rates (8–10), recipients receiving embryos originated from oocytes whose donors have received GnRH agonist have comparable pregnancy rates to recipients receiving embryos from hCG-matured oocytes (6, 7).

But, most relevant of all, these results were obtained without cycle coasting, cancellation, or OHSS in the donors ovulated with the GnRH agonist and are independent of E_2 serum levels. Moreover, the menses of the donors arrived 4 days later, and the ultrasound (1 week postretrieval) revealed a normal sized ovary. These observations give an idea of how powerful agonist-induced luteolysis is, but they should not be taken as an example or justification of extreme COH to maximize oocyte production.

These results, in our opinion, have important implications for the three pillars of the oocyte donation program: the donors, the recipients, and the physicians. For donors, the advantages of GnRH agonist ovulation are very straightforward. First of all, none of the donors who ovulated with the agonist developed any discomfort produced by or related to OHSS. Second, they recovered from the COH cycle in a very short while (menses returned after 4 days), giving them the possibility of resuming their studies or job in a short period of time, with no discomfort. And third, their attitude and feelings about donation are very positive, as they are willing to repeat and have recommended donation to their friends. For the recipients, the process of COH will be less stressful because there will be no delays from cycle cancellation or coasting, both of which are particularly upsetting and quite costly for a woman who has come to the clinic from a foreign country.

Finally, for the clinic and its staff directly involved in the oocyte donation program, GnRH agonist stimulation does not affect pregnancy rates but does avoid the complications (cancellation, coasting, and OHSS) that make ovulation uncertain. All this results in a friendlier atmosphere between donors and nurses, a less stressful COH, fewer phone calls and postretrieval ultrasound controls, probable reductions in clinic insurance costs, and, also, a potential increase in the number of donors. Furthermore, the disappearance of

OHSS from the donation process will greatly improve the image of this technique for many other physicians (endocrinologists, andrologists, etc.), the lay public, and the press (5). It is important to remember that in a society that is postponing maternity to the far limits of fecundity, oocyte donation is the only existing solution until other alternatives (in vitro oocyte maturation, for example) improve.

In conclusion, we would like to recommend that ovulation be triggered with a GnRH agonist instead of the classic hCG in IVF/oocyte donor cycles since good pregnancy rates can be obtained without cancellation, coasting, or OHSS. Thus, at the present time, these inconveniences have no more room in oocyte donation cycles.

Eleuterio R. Hernández, M.D., Ph.D.
José Luis Gómez-Palomares, M.D.
Elisabetta Ricciarelli, M.D.
Clínica FivMadrid, Madrid, Spain

REFERENCES

1. Garcia-Velasco JA, Isaza V, Quea G, Pellicer A. Coasting for the prevention of ovarian hyperstimulation syndrome: much ado about nothing. Fertil Steril 2006;85:547–54.
2. Sauer MV, Paulson RJ, Lobo RA. Rare occurrence of ovarian hyperstimulation syndrome in oocyte donors. Int J Gynaecol Obstet 1996;52:259–62.
3. Halme J, Toma SK, Talbert LM. A case of severe ovarian hyperstimulation in a healthy oocyte donor. Fertil Steril 1995;64:857–9.
4. ESHRE Task Force on Ethics and Law 12. Oocyte donation for nonreproductive purposes. Hum Reprod 2007;22:1210–3.
5. Covington SN, Gibbons WE. What is happening to the price of eggs? Fertil Steril 2007;87:1001–4.
6. Acevedo B, Gomez-Palomares JL, Ricciarelli E, Hernandez ER. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. Fertil Steril 2006;86:1682–7.
7. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Comparison of human chorionic gonadotropin and gonadotropin-releasing hormone agonist for final oocyte maturation in oocyte donor cycles. Fertil Steril 2007;88:237–9.
8. Humaidan P, Ejdrup Bredkjaer H, Bungum L, Bungum M, Grondahl ML, Westergaard L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum Reprod 2005;20:1213–20.
9. Kolibianakis EM, Schultze-Mosgau A, Schroder A, van Steirteghem A, Devroey P, Diedrich, et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocytes maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005;20:2887–92.
10. Balach J, Tur R, Creus, Buxaredas R, Fabregues F, Ballesca JL, et al. Triggering of ovulation by a gonadotropin release hormone agonist in gonadotropin-stimulated cycles for prevention of ovarian hyperstimulation syndrome and multiple pregnancy. Gynecol Endocrinol 1994;8:7–12.
11. Ricciarelli E, Sanchez M, Martinez M, Andres L, Cuadros J, Hernández ER. Impact of the GnRH antagonist in oocyte donation cycles. Fertil Steril 2003;79:1461–3.
12. The Practice Committee of the American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. Fertil Steril 2003;80:1309–14.
13. Kol SK. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fertil Steril 2004;81:1–5.
14. Tay CC. Use of gonadotropin-releasing hormone agonist to trigger ovulation. Hum Fertil (Cambridge) 2002;5:35–7.
15. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L, Brandes JM. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril 1991;56:213–20.

OBJETIVO 5

Demostrar que en los ciclos con antagonista de la GnRH, la inducción de la ovulación con un agonista de la GnRH no ocasiona hiperestimulación ovárica en pacientes con ovarios poliquísticos.

La inducción de la ovulación con un agonista de la GnRH en pacientes con ovarios poliquísticos no ocasiona hiperestimulación ovárica a pesar de niveles elevados de estradiol

Maria A Manzanares, Jose Luis Gómez-Palomares, Elisabetta Ricciarelli, Eleuterio R Hernández

Fertil Steril. 2010 Mar;93(4):1215-1219

Debido al alto riesgo que las pacientes con síndrome de ovarios poliquísticos (SOP) tienen de padecer una hiperestimulación ovárica en los tratamientos de FIV, realizamos un estudio con el fin de ofrecer a estas mujeres un tratamiento alternativo en el que las tasas de embarazo se mantuvieran sin riesgo de un cuadro de SHO en las pacientes que previamente habían tenido un ciclo de FIV cancelado por ese motivo (Manzanares et ál., 2010).

En FivMadrid habíamos observado que en los ciclos de donación de óvulos, ninguna de las donantes sufrió de un SHO (independientemente de los niveles de estradiol sérico) cuando la maduración final del oocito se realizaba con un agonista de la GnRH (Hernández et ál. 2009). Por eso planteamos la hipótesis de por qué no aplicar el mismo protocolo a las pacientes con síndrome de ovario poliquístico cuyos ciclos habían sido cancelados por síndromes de estimulación ovárica. La intención era doble. En primer lugar inducir el reclutamiento folicular y permitir la recuperación oocitaria sin el riesgo de desarrollar un síndrome de estimulación ovárica o una cancelación. En segundo, mantener unas tasas de embarazo aceptables permitiendo la congelación embrionaria y la transferencia en un ciclo posterior.

42 pacientes diagnosticadas de síndrome de ovario poliquístico se incluyeron en este estudio. Todas habían experimentado un ciclo de FIV previo que habían tenido que ser cancelado por riesgo elevado de síndrome de SHO (concentración de estradiol mayor a 3500 pg/ml y más de 20 folículos reclutados). En todos los casos, el tratamiento se había llevado a cabo siguiendo un protocolo largo con gonadotropinas y agonistas de la GnRH para frenar la hipófisis.

En el segundo ciclo (ciclo alternativo) el protocolo elegido fue estimular la foliculogénesis con FSH y frenar la actividad hipofisaria con un con un antagonista de la GnRH. En todos los casos las pacientes recibieron un tratamiento previo con anticonceptivos orales. Entre el tercer y cuarto día del ciclo menstrual se inició la estimulación ovárica con una dosis fija de entre 75 y 150 unidades al día de FSH recombinante. Esta estimulación se mantuvo durante cinco días. El protocolo de disminución paulatina de dosis se inició cuando los folículos sobrepasaron los 13 mm de diámetro. El día sexto de la estimulación se añadió el antagonista de la GnRH que se mantuvo hasta el día en que los folículos llegarán a los 18 milímetros y se programara la punción ovárica (independientemente de los niveles séricos de estradiol). La maduración final de los oocitos se realizó con un agonista de la GnRH (triptorelina) en lugar de hCG. De los embriones obtenidos después de la FIV/ICSI sólo se congelaron los de alta calidad.

El ciclo de preparación endometrial se realizó de la siguiente forma: el día 18 del ciclo menstrual se practicó una ecografía, para excluir patología ovárica y asegurar el reposo ovárico. Si esto se cumplía se administraba un agonista de la GnRH y después de la menstruación, se comenzaba con estradiol (Progynova, Bayer) en comprimidos administrados vía oral de forma creciente. Cuando el tamaño endometrial alcanzó los 8 mm se consideró apto para realizar la transferencia de los embriones criopreservados.

Tabla 1. La inducción de la ovulación con un agonista de la GnRH en pacientes con ovarios poliquísticos no ocasiona hiperestimulación ovárica a pesar de niveles elevados de estradiol. Parámetros basales, estimulación, reclutamiento y recuperación oocitaria.

	Primer ciclo	Segundo ciclo	
Número de pacientes	42	42	
Edad, años[a]	32,9 3,1	33,8 4,2	ns
Niveles basales de FSH, mUI/mL[a]	6 ± 2,58	6,2 ± 2,8	ns
Niveles basales de LH, mUI/mL[a]	6,1 ± 3,7	6 ± 3,4	ns
Niveles basales de Es, mUI/mL[a]	56 ± 33	44,1 ± 24,24	ns
Antagonista (ampollas)[a]	-	3,9 ± 1,4	-
Días de estimulación[a]	10,4 ± 2,1	9,78 ± 1,49	ns
FSH (UI)[a]	2259,66 ± 958	1943 ± 1158	ns
E2 (pg/ml) día final[a]	4809,6 ± 2947	4518.5±2118	ns
Folículos reclutados			
>18 mm [a]	13,2 ± 7,5	15,43± 7,3	ns
15-17 mm [a]	8,2 ± 6,7	9,1± 5,3	ns
Oocitos recuperados[a]	CANCELADO	12.6± 6.8	-
Síndrome de hiperestimulación ovárica	0	0	-
Oocitos en Metafase II, %	-	93	-
Oocitos en metafase I, %	-	2,2	-
Embriones grado 1, %	-	48	-
Embriones grado 2, %	-	30	-
Embriones grado 3, %	-	17	-
Embriones multinucleados,%	-	4,5%	-
Tasa de fecundación,%	-	69	-

[a]Media ± desviación estándar. Ns: No significativo.

La transformación del endometrio de proliferativo a secretor se consiguió con progesterona natural micronizada. El número de embriones transferidos dependió de los deseos del apareja, en todo caso nunca se transfirió más de tres embriones dado que es el

número máximo de de embriones que la Ley permite transferir en España.

No hubo diferencias significativas en cuanto a la edad niveles basales de FSH, LH o E2 entre el primer y segundo ciclo. En el segundo todas las pacientes consiguieron llegar a la punción ovárica sin necesidad de realizar "coasting" e independientemente de los valores del estradiol sérico. La media de oocitos recuperados fue de 12,6 ± 6,8 oocitos, 93% de los cuales se encontraban en fase MII (Tabla 1). Ninguna de las pacientes desarrolló síndrome de estimulación ovárica. El 33% de las pacientes que recibieron embriones congelados tuvieron una prueba de embarazo positiva (Tabla 2)

No hubo diferencias significativas en cuanto a la edad niveles

Tabla 2. La inducción de la ovulación con un agonista de la GnRH en pacientes con ovarios poliquísticos no ocasiona hiperestimulación ovárica a pesar de niveles elevados de estradiol. Gestaciones.

Número de ciclos	42	42
Embriones transferidos[a]	-	2,5 ± 0,6
Tasa de embarazo, n(%)	-	14/42 (33)
Gestaciones bioquímicas, n(%)	-	1/14(7)
Abortos, n(%)	-	1/13(7,6)
Gestaciones evolutivas, n(%)	-	12/14 (85)
Gestaciones gemelares, n(%)	-	2/14 (14,2)

[a]Media ± desviación estándar.

basales de FSH, LH o estradiol entre el primer y segundo ciclo. En el segundo, todas las pacientes consiguieron llegar a la punción ovárica sin necesidad de realizar "coasting" e independientemente de los valores del estradiol sérico. Ninguna de las pacientes desarrolló síndrome de estimulación ovárica. El 33% de las pacientes que recibieron embriones congelados tuvieron una prueba de embarazo positiva, Tabla 2 .

Por lo tanto, el uso del agonista de la GnRH en pacientes con SOP para provocar la ovulación ofrece un escenario totalmente distinto ya que independientemente de los valores hormonales y del número de folículos, las pacientes llegaron a tener un buen número de oocitos maduros, buenas tasas de fertilización y un buen número de embriones. Además ninguna de las pacientes desarrolló signos de hiperestimulación ovárica y todas estuvieron totalmente asintomáticas a los cuatro días de la punción.

En conclusión, la inducción de la ovulación con los agonistas de la GnRH tiene importantes ventajas para este tipo de pacientes ya que saben que se van a someter un ciclo en el cual no hay un riesgo elevado de hiperestimulación ovárica y que ofrece unas buenas tasas de embarazo en un ciclo de descongelación de embriones posterior.

Triggering ovulation with gonadotropin-releasing hormone agonist in in vitro fertilization patients with polycystic ovaries does not cause ovarian hyperstimulation syndrome despite very high estradiol levels

Maria A. Manzanares, M.D., Jose Lui Gómez-Palomares, M.D., Elisabetta Ricciarelli, M.D., and Eleuterio R. Hernández, M.D., Ph.D.

Clinica "FivMadrid," Juan Alvarez de Mendizabal, Madrid, Spain

Objective: To determine whether inducing ovulation with a GnRH agonist in patients with polycystic ovaries (PCO) would permit oocyte retrieval without the burden or risk of cancellation, coasting, or ovarian hyperstimulation syndrome (OHSS), thus maintaining pregnancy rates by allowing embryo cryopreservation for transfer in a subsequent cycle.
Design: Retrospective observational study.
Setting: Private institution.
Patient(s): Forty-two women who had previously experienced a controlled ovarian hyperstimulation (COH)/IVF cycle that had to be cancelled because of an elevated risk of OHSS.
Intervention(s): Forty-two PCO patients with a previous cancelled IVF cycle were assigned to a second controlled ovarian stimulation with recombinant FSH (75–150 IU/day) + GnRH antagonist (0.25 mg/day). Embryos were cryopreserved and transferred in a later cycle.
Main Outcome Measure(s): OHSS, oocyte retrieval, and pregnancy rates.
Result(s): In the first COH, the cycle had to be cancelled to avoid OHSS because E_2 serum levels were above safety levels (4809.6 ± 2947.7). However, in the second cycle (ovulation triggered with a GnRH agonist) and independent of E_2 serum levels (4518.5 ± 2118.85), all PCO patients eventually completed oocyte retrieval and frozen ET. With regard to pregnancy rates, 33% of patients receiving a transfer of a previously frozen embryo were successful. No patient developed OHSS.
Conclusion(s): Triggering ovulation with a GnRH agonist followed by embryo cryopreservation allows PCO patients to complete a COH/IVF cycle with no cycle cancellation, coasting, or OHSS and, finally, to attain good pregnancy rates. (Fertil Steril® 2010;93:1215–9. ©2010 by American Society for Reproductive Medicine.)

Key Words: PCO, OHSS, pregnancy, GnRH agonist, ovulation

Before the arrival of the GnRH antagonist, ovulation in IVF cycles had to be induced with a bolus of hCG. Because of its high affinity for and binding capacity to the LH receptor, hCG can induce complete final oocyte maturation, and it is well tolerated by most patients. Nevertheless, because of its long half-life and through a not well-understood mechanism, some patients may develop the hCG-dependent ovarian hyperstimulation syndrome (OHSS). Polycystic ovary (PCO) patients are at risk for OHSS, and it is calculated that approximately 1%–3 % of them may develop the syndrome (1–3).

A valuable indicator of OHSS risk is E_2 serum level. It is recommended that E_2 serum levels be determined during ovarian stimulation (1–3). Moreover, if E_2 circulating levels are above 3500 pg/mL (by the day hCG is indicated), it is necessary to start actions (coasting, withhold hCG, cycle cancellation) to prevent development of OHSS (1–3). Nevertheless, all these steps (cancellations, coasting, etc.) carry important sequelae (financial burden, stress, disappointment of cancelled oocyte retrieval) and leave such unpleasant memories in patients that some of them refuse to continue IVF treatment.

With this in mind, reproductive endocrinologists have constantly been looking for new approaches to avoid hCG-dependent OHSS in controlled ovarian hyperstimulation (COH) IVF cycles. One way is to trigger ovulation with a bolus of a GnRH agonist instead of hCG in COH/IVF cycles in which the GnRH antagonist has been used to suppress LH (4–9).

We have been routinely triggering ovulation with a GnRH agonist in oocyte donation cycles for the last 5 years and have obtained the same pregnancy rates as with hCG (7, 8). Furthermore, none of the donors have needed coasting or cancellation or developed OHSS (independent of E_2 serum level) after oocyte retrieval (7, 8).

Received October 12, 2008; revised November 27, 2008; accepted December 8, 2008; published online February 6, 2009.
M.A.M. has nothing to disclose. J.L.G.-P. has nothing to disclose. E.R. has nothing to disclose. E.R.H. has nothing to disclose.
Reprint requests: Eleuterio R. Hernández, M.D., Ph.D., Clínica de Medicina de la Reproducción y Ginecología "FivMadrid," c/Juan Álvarez de Mendizábal 74, 28008-Madrid, Spain (FAX: 34-915-610-700; E-mail: ehernandezm@fivmadrid.es).

Thus, we wondered why the same protocol could not be applied in patients with severe PCO who had already suffered cycle cancellation and OHSS. The approach would be able to achieve two goals. First, it could induce follicle recruitment and allow oocyte retrieval without the risk of cancellation, coasting, or OHSS. Second it could maintain pregnancy rates by allowing embryo cryopreservation and transfer in a subsequent cycle since embryo implantation capacity is unaffected when the agonist is used to trigger ovulation (7).

MATERIALS AND METHODS

Forty-two PCO patients were included in this retrospective observational study, following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on Human Research and with Institutional Review Board approval.

These high-risk patients exhibit PCO syndrome or a PCO morphology on ultrasound (3). All of the women had previously experienced a COH/IVF cycle, with a high response to gonadotropins, that had to be cancelled because of an elevated risk of OHSS (E_2 concentration $>$ 3500 pg/mL and $>$20 preovulatory and intermediate size follicles) on the day ovulation was indicated.

First COH Cycle

Forty-two PCO-patients were included in this group. As described elsewhere (10), on the 21st day of their menstrual period and after an ultrasound examination to exclude ovarian pathology, the GnRH agonist (Procrin, Abbot, Madrid, Spain) was given. Two weeks later, a dose of 75–150 units of recombinant FSH (Gonal F, Serono, Madrid, Spain; Puregon, Organon, Barcelona, Spain) were added for 5 days SC to induce folliculogenesis. Once the recruited follicles were $>$14 mm in diameter or E_2 serum levels were $>$300 pg/mL, a step-down protocol was initiated, and follicle development was monitored by sequential ultrasound scans. In the final evaluation, when the recruited follicles were $>$18 mm, final oocyte maturation (with hCG) and oocyte retrieval had to be cancelled to prevent OHSS because the E_2 serum levels were above 3500 pg/mL and there were too many intermediate sized follicles.

Second COH Cycle: Ovulation Triggered with GnRH Agonist

As described elsewhere (7, 8), patients were subjected to a step-down protocol in which LH activity was suppressed with a GnRH antagonist as follows. All patients received oral contraceptives (Etinilestradiol/Ciproterona Gineservice, Effik, Madrid, Spain) during the previous month. Beginning on the third or fourth day of their menstrual period, a fixed dose of 75–150 IU/day of recombinant FSH (Puregon, Organon; Gonal F, Serono) was given during 5 days to induce follicle recruitment. On day 6 of ovarian stimulation, 0.25 mg/day of the GnRH antagonist (Cetrotide, Serono; Orgalutran, Organon) was injected SC and repeated daily until the GnRH agonist (Decapeptil 0.1 mg, Ipsen Pharma, Barcelona, Spain) was given. Follicular recruitment and development were evaluated by ultrasound. If the follicles were $>$13 mm in diameter, a step-down protocol was initiated and follicle development was followed by sequential ultrasound scans. Once the recruited follicles were $>$18 mm in diameter and independent of E_2 serum levels final oocyte maturation was obtained with a single bolus of 0.2 mg/second of GnRH agonist (Decapeptil 0.1 mg/ampoule), the triggering dose of the agonist, before carrying out oocyte retrieval under sedation and ultrasound guidance 32–34 hours later.

Fertilization Procedure and Embryo Grade

Intracytoplasmatic sperm injection (ICSI) was routinely performed in all fertilization procedures, as described elsewhere (11). Fertilization was evidenced when two pronuclei were observed. Embryos were cultured until the day of transfer (day 3) in IVF media (Vitrolife, Mölndalsvägen, Göteborg) and graded (12).

Freezing and Thawing Procedure

Embryos were frozen on day 3 postretrieval for transfer at a later date. It is our policy to only freeze those embryos that at day 3 of development meet the transfer criteria (G1 and G2 embryos), all others are discarded. Cleavage-stage embryos were frozen in a sequential freezing solution (FREEZE-KIT 1, Vitrolife, Kunsgsbacka, Sweden) in a sterile multiwell dish (Nunc, Roskilde, Denmark). Briefly, embryos were placed in 400 μL of Cryo-PBS/2 minutes solution then transferred to 400 μL EFS1/10 minutes and, finally, placed in 400 μL of EFS2/10 minutes solution; they were then drawn by a 1-mL syringe into attached straws (Cryobiosystem, ĹAigle, France). The loaded and sealed straws were placed into a freezing chamber filled with liquid nitrogen (Nicool MS21, Air LiQUID, Madrid, Spain). Embryos were cooled slowly until reaching a final temperature of -196°C and then stored.

Embryos selected for transfer were thawed the night before the day planned for uterine transfer. They were placed in a commercial IVF-CCM sequential thawing solution (ThaW-Kit, Vitrolife) in a sterile multiwell dish (Nunc). Briefly, embryos were placed in 400 μL of TS1/5 minutes, then were transferred to 400 μL of TS2/5 minutes, placed in 400 μL of TS3/10 minutes, and, finally, placed in Cryo-PBS/5 minutes/room temperature (RT); they were then transferred to the CO_2 incubator to await transfer.

Endometrial Preparation and Pregnancy Confirmation

On the 21st day of their menstrual period and after an ultrasound examination to exclude ovarian pathology and to ensure ovarian quiescence, the GnRH agonist (Decapeptil 3.75 mg, Ipsen Pharma) was given. Endometrial development was induced with E_2 (Progynova, Shering, Barcelona, Spain) in all recipients as described elsewhere (7, 8). When the endometrial line evaluated by ultrasound scan was $>$8 mm, it was considered mature. To transform the endometrial phase to secretory, 600 mg/day of natural P (Progeffik, Effik) was given for at least 3 days before transfer.

TABLE 1

Baseline characteristics and outcome of ovarian stimulation.

Characteristic	First cycle	Second cycle	*P* value
No. of patients	42	42	
Age, y	32.9 ± 3.1	33.8 ± 4.2	NS
Serum levels on day			
FSH, mIU/mL	6 ± 2.58	6.2 ± 2.8	NS
LH, mIU/mL	6.1 ± 3.7	6 ± 3.4	NS
E_2, pg/mL	56 ± 33	44.1 ± 24.24	NS
GnRH antagonist, ampoules	—	3.9 ± 1.4	—
COH, d	10.4 ± 2.1	9.78 + 1.49	NS
FSH, IU	2259.66 ± 958	1943 ± 1158	NS
E_2 on day of hCG, pg/mL	4809.6 ± 2947.7	4518.5 ± 2118.85	NS
Recruited follicles, mm			
>18	13.2 ± 7.5	15.43 ± 7.3	NS
15–17	8.2 ± 6.7	9.1 ± 5.3	NS
Oocytes retrieved	Cancelled	12.6 ± 6.8	—
OHSS	0	0	—
Metaphase II oocytes, %	—	93	—
Metaphase I oocytes, %	—	2.2	—
Embryo grade, %			
G1	—	48	—
G2	—	30	—
G3	—	17	—
Multinucleated embryos, %	—	4.5	—
Fertilization rate	—	69	—

Note: Data are mean ± SD unless otherwise indicated. NS = not significant.

Manzanares. Triggering ovulation with GnRH agonist in PCO. Fertil Steril 2010.

The number of embryos thawed depends on the couple's desire regarding the number of embryos to be transferred. In any event, never more than three, since that is the number of embryos allowed in Spain. A pregnancy test (hCG; BioMerieux, Marcy l'Etoile, France) was performed 15 days after ET. If positive, an ultrasound scan was scheduled for 2 weeks later to determine the number and status of implanted embryos. The concurrency of a positive hCG and embryo(s) with a positive heart beat (seen by ultrasound) was defined as a clinical pregnancy; otherwise it was considered to be a biochemical pregnancy. Implantation rates were calculated by dividing the total number of embryos transferred by the number of embryos implanted.

Hormonal Assays

Hormonal assays used a commercial enzyme immunoassay kit (Vidas, BioMerieux, France). Basal values of FSH, LH, and E_2 were routinely determined before the initiation of ovulation induction in any given cycle (day 3–4).

Statistical Analysis

This is a retrospective, observational, nonrandomized study. We and the Institutional Review Board consider that randomization was not ethically appropriate because the population of patients selected for this study was at high risk for OHSS if hCG was used and because our pregnancy rates with fresh ETs when ovulation is triggered with the agonist are very low (16% pregnancy rates/cycle).

For descriptive statistics, we used means ± SD. Student's *t*-test, Fisher's exact test, and the χ^2-test were used where appropriate. Patients were recruited for the trial for only one ET. $P<.05$ was considered statistically significant.

RESULTS

As seen in Table 1, no significant differences were observed with respect to age (32.9 ± 3.1 vs. 33.8 ± 4.2 years) or basal serum levels of FSH (6 ± 2.5 vs. 6.2 ± 2.8 mUI/mL), LH (6.1 ± 3.7 vs. 6 ± 3.4 mUI/mL), and E_2 (56 ± 33 vs. 44.1 ± 24.2 pg/mL) in PCO patients between the first and the second IVF cycle, respectively.

Similarly, on the day ovulation was planned, no significant differences in regard to the length of COH (10.4 ± 2.1 vs. 9.78 ± 1.49 days), total units of FSH (2259.6 ± 958 vs. 1943 ± 1158), number of recruited follicles (>18 mm in diameter; 13.2 ± 7.5 vs. 15.43 ± 7.3), and E_2 serum levels (4809.6 ± 2947.7 vs. 4518.5 ± 2118.85 pg/mL) were seen

in PCO patients between the first and second IVF cycle, respectively (Table 1). Nevertheless, in the first cycle (in which ovulation required hCG), hCG had been withheld and oocyte retrieval cancelled to avoid OHSS because E_2 serum levels were above safety levels (Table 1).

However, in the second cycle in the same PCO patients and independent of E_2 serum level (4518.5 ± 2118.85 pg/mL), no patient was coasted and all of them underwent the oocyte retrieval procedure. A mean of 12.6 ± 6.8 oocytes were obtained, with 93% being in the meiosis II stage. After ICSI (69% fertilization rate), on day 3 of in vitro development, embryos were graded (48% G1, 30% G2, and 17% G3) and then frozen in liquid nitrogen.

With regard to pregnancy rates, 33% of patients receiving a thawed embryo (2.5 ± 0.6 embryos/patient) achieved pregnancy; 7.6% ended in miscarriage and 28% are ongoing pregnancies (Table 2). In respect to multiple pregnancies, 14.2% were twins and there were no triplets. No patient developed OHSS.

TABLE 2

Clinical results of frozen-thawed cycle.

	First cycle	Second cycle
No. of cycles	42	42
Embryos transferred, mean ± SD	—	2.5 ± 0.6
Pregnancy/transfer rate, n (%)	—	14/42 (33)
Biochemical pregnancies, n (%)	—	1/14 (7)
Miscarriages, n (%)	—	1/13 (7.6)
Ongoing pregnancies, n (%)	—	12/14 (85)
Twin pregnancies, n (%)	—	2/14 (14.2)

Manzanares. Triggering ovulation with GnRH agonist in PCO. Fertil Steril 2010.

DISCUSSION

The purpose of this study is to present an alternative treatment that maintains pregnancy rates in PCO patients whose previous IVF cycle and oocyte retrieval were cancelled because of a threat of OHSS. The PCO patients included in this study were very high responders with previous experience in COH/IVF cycles that ended in no retrieval, no transfer, frustration, and despair because of the possibility that OHSS would develop in subsequent COH/IVF cycles.

Since follicular recruitment in PCO patients is difficult to control, mainly due to excessive ovarian susceptibility to gonadotropin action, a different strategy was set in motion to maintain cycle progression, from ovulation induction to ET, and at the same time avoid the risk of cycle cancellation, coasting, or OHSS. To do this, PCO patients were subjected to a COH/IVF cycle in which LH was suppressed with a GnRH antagonist and ovulation triggered with a GnRH agonist (instead of hCG), a protocol that we have successfully tested previously (4–9). The difference with previous data is that to the best of our knowledge our paper is the first in the literature exploring the use of GnRH agonist in patients with severe PCO and E_2 levels (>5000 pg/mL) above established safety level recommendations (2); no patient developed OHSS.

There were no significant differences with respect to the age, basal hormone levels on day 3, total amount of FSH received, days of stimulation, number of recruited follicles, and E_2 serum levels (evaluated on the day ovulation was planned) between the first COH/IVF and second COH/IVF cycles. However, in their first cycle, PCO patients showed high E_2 levels that were indicative of possible OHSS, so hCG could not be administered and the planned oocyte retrieval was cancelled. Furthermore, these patients suffered from mild stimulation (abdominal distension, pain, enlargement of their ovaries), and until their ovaries recovered their normal size, they had to wait at least 2 months to start a new cycle. Having experienced these difficulties, the idea of a new ovarian stimulation can be distressing for these women.

Using the GnRH agonist to induce final oocyte maturation, the experience in the second COH/IVF cycle was quite to the contrary. First of all, and independent of E_2 serum levels and follicle number, oocyte retrieval was done, obtaining a good number of metaphase II oocytes, good fertilization rates, and, finally, a good number of embryos. Furthermore, none of the patients was coasted, not one developed OHSS, and they were all totally asymptomatic 4 days after oocyte retrieval. Based on our previous experience (>400 cycles with agonist), no special follow-up was needed (8). The patients reported to the clinic when menses occurred, no medication was prescribed, and no discomfort was reported. Similar results have been published in IVF (9) and oocyte donation (7, 8); they demonstrate that ovulation with a GnRH agonist is an excellent alternative when OHSS is a possibility and when a woman's desire for retrieval and ET is a priority.

All patients were informed that in our experience, as in that of others (13–15), pregnancy rates with fresh ETs agonist are low (16% pregnancy rate/cycle) when ovulation is triggered with GnRH. For that reason, we recommend that all embryos in a GnRH cycle be cryopreserved and transferred in a subsequent cycle, since agonists do not affect the embryo's implantation capacity (7, 16). In fact, pregnancy rates in cryothawed cycles were substantially better than in fresh transfer. In the former, 33% (14/42) of the patients become pregnant and none developed OHSS.

In this regard, it is important to consider and mention the promising results obtained with the same protocol by Engmann et al. using fresh ET; they report only reduced OHSS risk but excellent pregnancy rates in fresh ET in the same GnRH cycle (9). Nevertheless, in our experience (unpublished personal experience) with the standard combination of oral E_2 + vaginal P supplementation to maintain

endometrial receptivity for embryo implantation, this hormonal regime was unable to overcome luteolysis and allow implantation, as in many other studies (13–15). Since the patients in our study were supplemented with vaginal P instead of IM P (as in the Engmann et al. study), the route of P administration could partly explain the different results.

In summary, triggering ovulation with a GnRH agonist has important advantages for the patient and physicians. PCO patients know they will start a cycle in which they are not going to experience cycle cancellation, coasting, or OHSS and that, finally, offers the possibility of good pregnancy rates with a potential financial savings; all of these are positive factors. For physicians, the possibility of inducing folliculogenesis in high responding patients without the worry of OHSS or high E_2 levels and with the certainty of being able to perform an ET is also very positive.

REFERENCES

1. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update 2002;8:559–77.
2. Ovarian hyperstimulation syndrome. The Practice Committee of the American Society for Reproductive Medicine. Fertil Steril 2006;86: S178–83.
3. ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risk related to polycystic ovary syndrome. Fertil Steril 2004;81:19–5.
4. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L, Brandes JM. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril 1991;56:213–20.
5. Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report. Short communication. Hum Reprod 2000;15:1965–8.
6. Kol SK. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fertil Steril 2004;81:1–5.
7. Acevedo B, Gomez-Palomares JL, Ricciarelli E, Hernández ER. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. Fertil Steril 2006;86: 1682–7.
8. Hernández ER, Gomez JL, Ricciarelli E. No room for cancellation, coasting or ovarian hyperstimulation syndrome in oocyte donation cycles. Fertil Steril. Published online 12 June 2008 [Epub ahead of print].
9. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. Fertil Steril 2008;89:84–1.
10. Gómez-Palomares JL, Acevedo-Martín B, Andrés L, Ricciarelli E, Hernández ER. LH improves early follicular recruitment in women over 38 years old. Reprod Biomed Online 2005;11:409–14.
11. Palermo GP, Joris H, Devroey P, Van Steirteghem AC. Pregnancy after intracytoplasmatic injection of a single spermatozoon into an oocyte. Lancet 1992;340:17–8.
12. Veeck LL. Atlas of Human Gametes and Conceptuses. New York: Parthenon Publishing Group, 1999.
13. Humaidan P, Ejdrup Bredkjaer H, Bungum L, Bungum M, Grondahl ML, Westergaard L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum Reprod 2005;20:1213–20.
14. Kolibianakis EM, Schultze-Mosgau A, Schroder A, van Steirteghem A, Devroey P, Diedrich K, et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocytes maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005;20:2887–92.
15. Griesinger G, Diedrich K, Devroey P, Koliobinakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update 2006;12:159–68.
16. Griesinger G, Koliobinakis EM, Papanikolaou EG, Diedrich K, Van Steirteghem A, Devroey P, et al. Triggering of final oocyte maturation with gonadotropins-releasing agonist or human chorionic gonadotropins. Live birth after frozen-thawed embryo replacement cycles. Fertil Steril 2007;88:616–21.

Discusión

Antagonistas de la GnRH en los ciclos de inseminación artificial.

En las estimulaciones ováricas destinadas a realizar una inseminación artificial, los antagonistas de la GnRH han permitido prolongar el desarrollo folicular, disminuir el riesgo de una luteinización precoz y aumentar el número de folículos presentes el día de la inseminación (Gómez-Palomares et ál. 2005; Gomez-Palomares et ál. 2008). Todo ello ha aumentado los porcentajes de éxito en este tipo de tratamientos. Pero también puede haber inconvenientes. El hacer que el número de folículos maduros sea mayor conlleva el aumentar el riesgo de gestaciones múltiples, la principal complicación de los tratamientos de reproducción asistida. Como se observó en nuestro primer estudio sobre antagonistas en ciclos de IA (Gómez-Palomares et ál. 2005) en el grupo antagonista hubo un mayor número de folículos ≥16 mm (2,4±1,4 folículos frente a 1,7±1,2 folículos) lo que llevó a que en el grupo antagonista hubieran más gestaciones gemelares e incluso una triple.

Otros autores proponen el uso de los antagonistas de la GnRH en ciclos de IA como forma de evitar que éstas ocurran durante los fines de semana, algo que en determinados ámbitos de trabajo supone un inconveniente, (Matorras et ál. 2006). A priori esto puede resultar chocante pero éticamente supone más problema el cancelar un ciclo al no disponer de personal para atender a la mujer durante los fines de semana que postergar la inseminación con la ayuda de los antagonistas de la GnRH.

Aunque en nuestros ciclos de IA no medimos la LH durante el tratamiento, se ha descrito que en un ciclo de IA en el que no se use un antagonista de la GnRH el riesgo de que ocurra un escape de LH con la consiguiente luteinización precoz es del 28,2%, en cambio el uso de los antagonistas lo reduce al 5,3% (Kosmas et ál. 2008). Esto supone otra ventaja añadida dado que la cancelación de

un ciclo provoca un aumento del coste de los tratamientos de reproducción asistida.

Con la idea de ahondar más en el uso de los antagonistas de la GnRH en este tipo de tratamientos diseñamos nuestro trabajo publicado en 2008. En él demostramos que sólo en los ciclos en los que se habían reclutados varios folículos valía la pena usar el antagonista de la GnRH como terapia adyuvante (Gomez-Palomares et ál. 2008). En los ciclos monofoliculares el coste económico del ciclo aumentaba sin que si viera reflejado en un aumento de las posibilidades de éxito.

Antagonistas de la GnRH en los ciclos de donación de oocitos.

La disponibilidad de los antagonistas de la GnRH ha supuesto una revolución en algunos tratamientos de reproducción asistida, por ejemplo los ciclos de donación de oocitos. Su uso permite elegir como inductor final de la maduración oocitaria bien la hCG, más arriesgado, bien un agonista de la GnRH, menos. Y gracias a investigaciones como las presentadas aquí, el síndrome de hiperestimulación ovárica ha desaparecido por completo en las donantes de oocitos (Acevedo et ál. 2006; Hernández et ál. 2009).

En nuestro trabajo de 2006 en este área estudiamos si la calidad embrionaria se comprometía al usar un agonista de la GnRH en lugar de hCG en los ciclos de donación de oocitos (Acevedo et ál. 2006). Los resultados mostraron que no y que además las donantes se sometían a un protocolo mucho más seguro en el que no se dio ni un solo caso de síndrome de hiperestimulación ovárica. Además su recuperación fue mucho más rápida al acortarse la fase lútea considerablemente de 13,63 ± 2,1 días en el grupo hCG a 4,1 ± 0,70 en el grupo agonista. Este estudio fue uno de los primeros que marcaron la pauta a seguir en

cuanto a la forma en la que se debe estimular a una donante para que, sin perder las opciones de embarazo en la receptora, se minimice el riesgo de complicaciones.

Ya centrándonos en comparar el protocolo estándar hasta la fecha (maduración final con hCG) con el que nosotros proponíamos como más seguro para la donante (maduración con agonista de la GnRH en un ciclo con antagonista de la GnRH) realizamos el estudio publicado en 2009 (Hernández et ál. 2009). Se revisaron 429 ciclos, 119 ciclos en el que protocolo realizado había sido el clásico con hCG y 176 en los que se había optado por el agonista de la GnRH. En el grupo hCH ocurrieron un 3,2% de hiperestimulaciones ováricas y además se tuvieron que cancelar el 1,6% de los ciclos por el riesgo de que esa complicación apareciera. Nada de todo esto ocurrió en el otro grupo. Quedaba patente que el nuevo protocolo era mucho más seguro y de hecho es el estándar hoy en las estimulaciones de las donantes de oocitos.

Antagonistas de la GnRH en los ciclos de FIV.

Un número considerable de mujeres padece alguna variante del síndrome del ovario poliquístico. Se calcula que del 5 al 10% de las mujeres en edad fértil lo padecen (Amato & Simpson 2006). El riesgo de alta respuesta tras una estimulación con gonadotropinas en este tipo de pacientes es mayor. Incluso superior al que presentan las donantes de oocitos . La llegada de los antagonistas en la primeros años de este siglo ha permitido que existan otras opciones a la hora de realizar la estimulación ovárica y la inducción final de la ovulación. El trabajo que aquí presentamos se centró en aquellas mujeres en las que previamente se había cancelado un ciclo de FIV (Manzanares et ál. 2010). Esto supone siempre un drama para la pareja, a parte del coste económico. La frustración que ocasiona es inmensa al no poderse llegar ni siquiera a culminar

el tratamiento creándose al menos embriones para ser posteriormente transferidos.

Al utilizar, en el contexto de un protocolo con antagonista de la GnRH, un agonista de la GnRH, se abolió por completo el riesgo de hiperestimulación ovárica. De hecho en nuestro trabajo ninguna de las pacientes lo padeció o tuvo que someterse a alguna estrategia para reducir el riesgo de que apareciera. Las posibilidades de conseguir un embarazo si se transfieren los embriones en el mismo ciclo en el que se ha usado el agonista de la GnRH como inductor final de la ovulación son bajas (Griesinger et ál. 2005). Por ello se informó a todas las pacientes de que era más conveniente congelar los embriones y transferirlos en un ciclo posterior. Ya habías demostrado en un trabajo anterior (Acevedo et ál. 2006) que la calidad embrionaria no se ve afectada por el uso del agonista. Los resultados en el ciclo de descongelación fueron excelentes. Se consiguió un 33% de tasa de embarazo en un grupo de mujeres en las que ya se había cancelado su primer ciclo de FIV y por tanto sin haber tenido ninguna opción.

Conclusiones

1. El uso de los antagonistas de la GnRH aumenta de forma significativa las tasas de embarazo en los ciclos IA.

2. El aumento de las tasa de embarazo evidenciados en los ciclos de IA con el uso de los antagonistas de la GnRH sólo se produce si los ciclos son multifoliculares y no en los monofoliculares.

3. Los agonistas de la GnRH pueden ser utilizados como inductores de la maduración final oocitaria en los ciclos en los que se usan los antagonistas de la GRH, sin que afecten la calidad embrionaria.

4. En los ciclos de donación de oocitos el único protocolo recomendable es aquel en el que se usa primero un antagonista de la GnRH y después un agonista de la GnRH ya que evita el riesgo de hiperestimulación en la donante.

5. Las pacientes con SOP se benefician de un protocolo secuencial de antagonistas/agonistas de la GnRH y criopreservación de embriones, a fin de evitar el SHO y mantener las tasas de embarazo.

Bibliografía

Acevedo B, Gomez-Palomares JL, Ricciarelli E, Hernández ER. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. Fertil Steril 2006;86:1682–7.

Allegra A, Marino A, Coffaro F, Scaglione P, Sammartano F, Rizza G, Volpes A. GnRH antagonist-induced inhibition of the premature LH surge increases pregnancy rates in IUI-stimulated cycles. A prospective randomized trial. Hum Reprod 2007;22:101–8.

Amato P, Simpson J-L. Genética del síndrome de ovarios poliquísticos. EMC -Ginecología-Obstetricia 2006;140-A-20:1–7.

Coccia ME, Comparetto C, Bracco GL, Scarselli G. GnRH antagonists. European Journal of Obstetrics & Gynecology and Reproductive Biology 2004;115:44–56.

Devroey P, Pados G. Preparation of endometrium for egg donation. Hum Reprod Update 1998;4:856–61.

Fares FA, Suganuma N, Nishimori K, LaPolt PS, Hsueh AJ, Boime I. Design of a long-acting follitropin agonist by fusing the C-terminal sequence of the chorionic gonadotropin beta subunit to the follitropin beta subunit. Proc Natl Acad Sci USA 1992;89:4304–8.

Gomez-Palomares JL, Acevedo-Martín B, Chávez M, Manzanares MA, Ricciarelli E, Hernández ER. Multifollicular recruitment in combination with gonadotropin-releasing hormone antagonist increased pregnancy rates in intrauterine insemination cycles. Fertil Steril 2008;89:620–4.

Gómez-Palomares JL, Juliá B, Acevedo-Martín B, Martínez-Burgos M, Hernández ER, Ricciarelli E. Timing ovulation for intrauterine insemination with a GnRH antagonist. Hum Reprod 2005;20:368–72.

Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update 2005;12:159–68.

Hernández ER. Disfunción reproductiva: una definición global de la infertilidad para el siglo 21. Revista iberoamericana de fertilidad 2011;3.

Hernández ER, Gomez-Palomares JL, Ricciarelli E. No room for cancellation, coasting, or ovarian hyperstimulation syndrome in oocyte donation cycles. Fertil Steril 2009;91:1358–61.

Huirne, J.A. and Lambalk, C.B. Gonadotropin-releasing-hormone-receptor antagonists. *Lancet 2001;* 358 :1793–1803.

Kaplan PF, Katz SL, Thompson AK, Freund RD. Cycle fecundity in controlled ovarian hyperstimulation and intrauterine insemination. Influence of the number of mature follicles at hCG administration. J Reprod Med 2002;47:535–9.

Kosmas IP, Tatsioni A, Kolibianakis EM, Verpoest W, Tournaye H, Van der Elst J, Devroey P. Effects and clinical significance of GnRH antagonist administration for IUI timing in FSH superovulated cycles: a meta-analysis. Fertil Steril 2008;90:367–72.

Lunenfeld B. Historical perspectives in gonadotrophin therapy. Hum Reprod Update 2004;10(6):453–67.

Manzanares M, Gomez-Palomares JL, Hernández ER. Triggering ovulation with gonadotropin-releasing hormone agonist in in vitro fertilization patients with polycystic ovaries does not cause ovarian hyperstimulation syndrome despite very high estradiol levels. Fertil Steril 2010;93:1215–9.

Martínez L. Prints en Fertilidad. Estimulante carrera hacia la fertilidad. Barcelona: Ediciones Mayo, 2011.

Matorras R, Ramón O, Expósito A, Corcóstegui B, Ocerin I, Gonzalez-Lopera S, Rodríguez-Escudero FJ. Gn-RH antagonists in intrauterine insemination: the weekend-free protocol. J Assist Reprod Genet 2006;23:51–4.

Millar RP. Gonadotropin-Releasing Hormone Receptors. Endocrine Reviews 2004;25:235–75.

Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 1999;14:698–703.

O'Dowd MJ, Philipp EE. The History of Obstetrics and Gynecology. London: Parthenon Publiching, 2000.

Ragni G, Alagna F, Brigante C, Riccaboni A, Colombo M, Somigliana E, Crosignani PG. GnRH antagonists and mild ovarian stimulation for intrauterine insemination: a randomized study comparing different gonadotrophin dosages. Hum Reprod 2004;19:54–8.

Ragni G, Caliari I, Nicolosi AE, Arnoldi M, Somigliana E, Crosignani PG. Preventing high-order multiple pregnancies during controlled ovarian hyperstimulation and intrauterine insemination: 3 years' experience using low-dose recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. Fertil Steril 2006;85:619–24.

Raju TN. The Nobel chronicles. 1977: Roger Charles Louis Guillemin (b 1924); Andrew Victor Schally (b 1926); Rosalyn S Yalow (b 1921). Lancet 1999;23:1481.

Speroff, L. & Fritz, M.A. *Endocrinología Ginecología Clínica y Esterilidad*. Madrid: Wolters Kluwer Helth, 2006.