P-135 Etiology of female infertility as prognostic factor for oocyte number and quality

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Introduction: Ovarian reserve is influenced by age, different medical conditions and exogenous factors. The quality of a woman's eggs is directly related to fertility and is crucial to conception. Poor egg quality may lead to **infertility** and repeated miscarriages. The aim of our study was to examine weather medical conditions such as PCOS, endometriosis and tubal problems influence the number and egg quality.
Material and Methods: Retrospective study included 355 women who underwent assisted conception treatment between January 2008 and December 2009 in university-affiliated clinic in Belgrade. Patients were divided in relation to age (< 25, 26-30, 31-35, 36-38 years) and different etiology of female infertility: 151 patients with tubal factor, 21 patients with endometriosis, 21 patients with PCOS and 162 patients with unknown etiology. Groups of same age were compared regarding oocyte number and quality. Where ICSI was performed evaluation of quality was assessed after enzymatic denudation. MII phase oocytes with no degenerative signs were considered of a good maturity and quality and MI, germinal vesicle stage (GV) and degenerated cells (Deg) were considered of a bad maturity and quality. Where IVF was performed the good quality cells are the ones that have been fertilized. To reduce the effect of paternal factors we accepted only patients with good sperm parameters. Kruskal Wallis ANOVA test has been used to compare different groups of patients. $P < 0.05$ was considered significant.
Results: Three thousand one hundred and fifty retrieved oocytes from 355 women, divided into four age groups, were subjected to evaluation of quality. Statistical analysis of group aged < 25 was not possible due to insufficient number of patients (n = 8). There was no significant difference regarding number and oocyte quality in groups aged 26-30 and 36-38 between patients with different etiological factor ($P > 0.05$). However, Kruskal Wallis test showed highly significant difference ($P < 0.01$) in group of women aged 31-35, for both observed parameters. Patients diagnosed with PCOS, endometriosis, tubal problems and patients of unknown etiology had significantly different number of retrieved oocytes (14.1 ± 1.8; 6.2 ± 1.1; 9.7 ± 1.1; 8.0 ± 0.5 respectively, $P = 0.004$) and number of good quality oocyte (8.6 ± 1.3; 4.2 ± 0.9; 6.0 ± 0.6; 4.7 ± 0.3 respectively, $P = 0.006$). The mean number of oocytes retrieved in patients with PCOS was significantly higher, whereas patients with endometriosis had significantly lower number of retrieved oocytes ($P = 0.004$). The similar trend was observed analyzing oocyte quality ($P = 0.006$). Endometriosis had significant impact on oocyte quality, reducing the number of good quality oocytes.
Conclusions: Our results support an association between certain medical conditions and ovarian reserve. PCOS and endometriosis influence the number and egg quality and therefore could be used as prognostic factors of great value.

P-136 In vitro maturation does not affect the geometry of the metaphase II spindle and cortical actin polarization of human oocytes

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Introduction: Different lines of evidence suggest that in vitro maturation (IVM) may influence the organization of the metaphase II (MII) spindle of mammalian oocytes. For example, in the mouse in vivo matured (IVO) oocytes exhibit small spindles with focused poles, whereas oocytes matured under inappropriate culture conditions display a large barrel-shaped spindle with flat poles. This could have implications for chromatid segregation and conservation of ooplasmic components required for pre-implantation development. The goal of the present study was to evaluate whether IVM can affect the morphology of the MII spindle of human oocytes.
Material and Methods: Oocytes were obtained from consenting IVF patients undergoing controlled ovarian stimulation. Within 2 hours from recovery, cumulus cells were removed and surplus oocytes were destined to different treatments depending on their stage of development. MII oocytes were immediately fixed and stained to detect chromatin, alpha- and beta-tubulin and actin by fluorescence confocal microscopy. Immature germinal vesicle (GV) oocytes were placed in microdrop cultures of IVM medium (Origio, Denmark) at 37°C in a humidified atmosphere of 5% CO_2 and 5% O_2. After 30 hours, oocytes that matured to MII were fixed and stained as described above. Labelled oocytes were analysed using a Leica TCS SP2 Laser Scanning Confocal microscope and a complete z-series for each spindle was collected at 0.3 µm intervals and reconstructed as a three-dimensional image. Spindles were analysed according to one- and two-dimensional characteristics. Chromosomes were classified as scattered or aligned at the metaphase plate.
Results: Eighteen IVO and ten in-vitro matured oocytes were analysed. The MII spindles of these two groups were morphologically equivalent as shown by comparing pole-to-pole axis (11.35 ± 1.09 µm in IVO oocytes vs. 11.31 ± 2.10 µm in IVM oocytes), equatorial axis (8.94 ± 1.18 µm in IVO oocytes vs. 8.88 ± 2.03 µm in IVM oocytes), the ratio of these dimensions (1.28 ± 0.15 µm in IVO oocytes vs. 1.35 ± 0.43 µm in IVM oocytes), and area of maximum projection (83.98 ± 11.60 µm^2 in IVO oocytes vs. 84.88 ± 32.98 µm^2 in IVM oocytes). Moreover, the two spindle categories were not different with respect to the difference in the distances of the MII plate from the pole near the cortex and the opposite pole. This difference was -0.54 ± 1.21 µm vs. -0.31 ± 1.28 µm in IVO and IVM oocytes, respectively. Analysis on a possible association between chromosome scattering from the MII plate and the above described morphological parameters revealed a positive linear correlation between the number of displaced chromosomes and the ratio between pole-to-pole and equatorial axes ($P = 0.039$). The percentage of oocytes showing spindles with aligned chromosomes was low (16.67%, 3/18, in IVO group; 20.00%, 2/10, in IVM group), probably as a consequence of the advanced patient age (≥ 35 years). Finally, it was observed that the intensity of the signal of cortical actin near the spindle was significantly higher in comparison to the opposite cortical position, in both IVO (155.18 ± 84.40, proximal actin vs. 118.00 ± 70.05, distal actin) and IVM oocytes (198.42 ± 71.33, proximal actin vs. 114.71 ± 34.24, distal actin).
Conclusions: These preliminary observations suggest that IVM conditions do not alter the geometry of MII spindle of human oocytes. In addition, it emerges that the MII plate is not equidistant from to the poles but is shifted towards the one nearest the cortex. Finally, distribution of cortical actin appears to be polarized, perhaps to ensure localization and anchorage of the spindle. Futures studies are warranted to confirm these preliminary data and better understand the relationship between spindle morphology and function.

P-137 Changes in oolemma retardance and cortical actin during meiotic maturation in human oocytes

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Introduction: Little is known about human oocyte cytoplasmic maturation, a process that occurs simultaneously with nuclear maturation from GV to MII stages. Under polarized light microscopy oocyte oolemma displays a characteristic birefringence which could be related to the cortical actin cytoskeleton projecting within the oocyte microvilli. The density and shape of oocyte microvilli undergoes a remarkable rearrangement during transition from GV to MII. Main aims of this study are 1) to evaluate oolemma birefringence in immature and mature oocytes collected after ovarian stimulation in ICSI cycles; 2) to explore the feasibility of oolemma analysis with polarized light microscopy as a biomarker for non-invasive visualization of subcellular structures in human oocytes; 3) to understand whether the oolemma birefringence is due to the ordered assembly of actin microfilaments within the oocyte cortex.
Material and Methods: Polarized light microscopy (Spindle View, RI) was used to study oolemma birefringence in different nuclear maturation stages. Oolemma retardance was evaluated recording the median birefringence of twelve cords of 2µm perpendicular to the plasma membrane for each oocyte. Eight GV, MI and MII oocytes were first imaged at the Spindle-view and then fixed and labeled with rodhamine phalloidin for confocal laser scan microscope (CLSM) analysis. Oolemma retardance data were analyzed using ANOVA with

post-hoc Bonferroni's correction and Pearson's X2 correlation was used to compare Polscope and CLSM data.
Results: A total of 50 oocytes for each maturation stage GV, MI and MII from 45 patients were imaged and oolemma birefringence was recorded. GV oolemma mean retardance was 1,65 nm (95% CI 1.53 to 1.76), while mean retardance of MI and MII oocytes was 2.32 nm (95%CI 2.1 to 2.53) and 1.99 nm (95%CI 1.64 to 2.35), respectively. In particular, oolemma retardance in MII oocytes showed a high heterogeneity with values ranging from 1.01 nm to 4.28 nm. Moreover 15 GV were imaged sequentially during in vitro maturation and a significant increase in retardance was observed. ANOVA analysis with Bonferroni's post hoc correction showed that mean GV oolemma retardance was significantly different when compared either with MI than MII oocytes (p = o. oo1 and 0.04 respectively), whereas mean MI and MII retardance were not different. Oolemma birefringence and F-actin fluorescence showed a significant linear correlation (R^2 = 0,835 Pearson's X^2 p = 0.01). Data indicate that oolemma retardance is due to the ordered arrangement of cortical actin within microvilli.
Conclusions: Our data suggest for the first time that human oocyte oolemma birefringence analysis is related to nuclear maturity and reflects microvillar actin organization and density. Therefore, oolemma imaging is potentially a new biomarker for the non-invasive visualization of subcellular structures in human oocytes.

P-138 The developmental capacity of human in vitro matured oocytes retrieved from small antral follicles (4-6 mm) in non-hCG-primed cycles

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Introduction: Priming with human chorionic gonadotrophin (hCG) before oocyte retrieval is a common strategy in oocyte *in vitro* maturation (IVM) treatment. Although this approach may have a beneficial effect on the number of oocytes retrieved and on oocyte maturation rates, hCG may disrupt important signaling processes at the oocyte-somatic cell interface during oocyte maturation *in vitro*, and there is no strong evidence that hCG triggering leads to higher pregnancy rates in patients with polycystic ovary syndrome (PCOS). In these patients, an important number of cumulus-oocyte complexes (COCs) obtained during oocyte retrieval are aspirated from small antral follicles with a mean diameter between 4 and 6 mm. We investigated whether oocytes derived from these small antral follicles can produce embryos that implant after maturation in an IVM system without hCG priming.
Material and Methods: Between January and December 2010, 96 IVM-treatment cycles were performed at UZ Brussel in patients with PCOS. Patients were administered an average cumulative dose of 510 ± 190 IU uFSH or HP-HMG daily before oocyte retrieval. When endometrial thickness was at least 5 mm, the oocyte retrieval was scheduled on the following day. No hCG trigger was given. In 33 of these cycles, all follicles were between 4 and 6 mm diameter and no dominant follicle was present at the moment of oocyte retrieval. COCs were retrieved and cultured in IVM culture medium suite (ORIGIO) for 38 hrs at 5% CO_2 and 20% O_2. After IVM, oocytes were denuded and ICSI was performed at one time point (one hour after denuding). Maturation rate, *in vitro* production of embryos and clinical outcomes after embryo transfer were evaluated.
Results: The mean age of the patients was 28.5 ± 3.9 years. All COCs were retrieved from follicles with a diameter between 4 and 6 mm. In total, 587 COCs (with an average of 17.8 ± 10.2 COCs / cycle) were obtained, from which 287 (48.9%) were at metaphase II (average 8.7 ± 5.3 COCs / cycle) after 38 hours of IVM. The mean fertilisation rate was 63% (average 5.6 ± 3.3 / cycle). On day 3 after ICSI, 50% of embryos (average 2.9 ± 2.3 / cycle) had a good morphological quality (more than 6 cells and classified as grade 1 or 2) and were eligible for transfer. In five cycles (15%), no embryo transfer was performed due to poor embryo quality. In 28 cycles, an embryo transfer was performed on day 3 after ICSI, with an average of 1.29 ± 0.46 embryos per transfer. Four ongoing pregnancies (clinical pregnancy rate of 14.2%) were established and two healthy babies were born.
Conclusion: PCOS patients represent a heterogeneous group. In a subset of these patients, follicular development is arrested at the small antral follicular stage, and follicles do not grow beyond 6 mm diameter despite uFSH or HP-HMG priming in IVM treatment cycles. In an IVM system without hCG-priming before oocyte retrieval, the oocytes derived from these small follicles can complete meiosis *in vitro*, be fertilised, and develop into healthy offspring. Further research is needed to optimise the clinical and laboratory protocol of IVM treatment, tailored to the specific follicular constellation of the ovary in patients with PCOS.

P-139 Isolated teratozoospermia patients may not necessarily benefit from intracytoplasmic sperm injection treatment

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Introduction: Teratozoospermia has been reported to affect in vitro fertilization (IVF) outcome, and intracytoplasmic sperm injection (ICSI) has been considered as a possible treatment for those with male factor infertility including teratozoospermia. However, there has been a report asserting that there is no relationship between isolated teratozoospermia and outcome, and thus no benefit from ICSI treatment (Keegan et al., 2007). The most recent meta-analysis also concludes that IVF with or without ICSI yields similar clinical pregnancy rate in isolated teratozoospermia population (Hotaling et al., 2010). Our study aimed to compare clinical outcomes of Korean couples with isolated teratozoospermia which is defined as normal morphologic forms being less than 5% with all the other normal semen parameters.
Material and Methods: A retrospective analysis of 289 cycles of IVF/ICSI were divided into two groups, 244 cases of normozoospermia (> 4% normal morphology) and 46 cases of isolated teratozoospermia (≤ 4% normal morphology), according to strict morphology using Kruger/Tygerberg strict criteria. The fertilization rate was taken as the primary outcome.
Results: There was no difference in age of patient and partner, BMI, and basal FSH, used gonadotropin dose, days of stimulation, $E_{2,}$ number of oocytes retrieved and embryos transferred between normozoospermia and isolated teratozoospermia group. However sperm motility of normozoospermia was significantly higher than that of isolated teratozoospermia (72.9 ± 15.4 vs. 67.0 ± 11.3, $p < 0.01$). The number of grade I, II and top quality embryos were similar between the two groups (0.7 ± 0.9 vs. 0.9 ± 0.9; 0.5 ± 0.7 vs. 0.5 ± 0.7). Isolated teratozoospermia group was then divided into ICSI and conventional IVF groups. The two groups had similar clinical characteristics, as well as fertilization rate, number of grade I, II and top quality embryos (45.6% vs. 62.2%; 0.8 ± 0.7 vs. 1.1 ± 1.1; 0.5 ± 0.7 vs. 0.5 ± 0.6).
Conclusions: Isolated teratozoospermia patients may not necessarily benefit from ICSI treatment. Isolated teratozoospermia does not seem to affect IVF success according to our study. For isolated teratozoospermia couples, conventional IVF can be considered as an option as well.

P-140 A novel immortalized human cumulus cells lines with capacity to support human embryonic stem cells growthing: clinical applications

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Introduction: Cumulus cells (CCs) that surrounding oocyte are biologically distinct from other follicular cells and perform specialized roles, transmitting signals within the follicles and supporting oocyte growth during follicular development. The aim of this study was, to generate CCs lines by a robust protocol and to evaluate the ability of these feeder layers to support growth of human embryonic stem cells (hESCs) and to retain the stemness and pluripotency of hESCs.
Material and Methods: Cumulus-oocyte complexes (COCs) were collected from women undergoing in vitro fertilization. CCs were dissociated mechanically from the oocyte and adapted to growth on a human collagen substrate in a defined medium for a long period. The immortalization of CCs lines was performed by infection with retrovirus vector pEGN-hTERT (human telomerase reverse transcriptase). Affymetrix Human Genome U133 Plus 2.0 DNA chips were performed to establish the global gene expression in CCs lines at different passages (P3, P6 and P9) and protein expression was analyzed by