activation by triggering a long lasting series of oscillations in the cytosolic free Ca2+ concentration. These so called Ca2+ oscillations are driven by production of inositol 1,4,5-trisphosphate (InsP3). We have discovered that mammalian sperm contain a sperm specific isoform of phospholipase C, called PLCzeta that can generate InsP3. Introduction of PLCzeta into mouse oocytes can trigger sustained Ca2+ oscillations and development up to at least the blastocyst stage. We have also shown that injection of PLCzeta cRNA causes Ca2+ oscillations in human oocytes. It is currently unclear how PLCzeta causes Ca2+ oscillations but it may interact with specific factors in the oocyte cytoplasm. We have studied the effects of PLCzeta on development using luciferase tagged constructs. We can thereby measure both PLCzeta expression and Ca2+ oscillations in oocytes during the activation process. Studies using luciferase constructs in both mouse and human oocytes indicates that, whilst oocyte activation can occur after introduction of a wide range of PLCzeta concentrations, there is a narrow range of concentrations that is compatible with development to the blastocyst stage. It is possible that a lack of, or inappropriate level of PLCzeta in sperm may explain some cases of male factor infertility.

O-145  Sperm selection for ICSI at high magnification: IMSI
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During the last decade, there has been a growing interest on the development of new techniques that improve the preparation and visualization of sperm to better select those spermatozoa that can support embryo development to the birth of a healthy baby.

The introduction of a new concept called “motile-sperm organellar-morphology examination” (MSOME), permits to examine motile spermatozoa in real time. At identical magnification, the use of Nomarski differential interference contrast optics let to observe more precisely minimal structural defects in the head of spermatozoa, such as vacuoles as compared with the Hoffman Modulation Contrast.

The combination of MSOME and ICSI is called "intracytoplasmic morphologically selected sperm injection" (IMSI).

The literature shows that selection of spermatozoa showing normal nuclear shape using IMSI is positively associated with pregnancy rates (PR) after day 3 embryo transfers (ET) in couples with previous implantation failures and in patients with an elevated degree of DNA fragmented spermatozoa.

Despite no apparent difference in embryo development to day 3, the existence of spermatozoa of normal nuclear shape but with large vacuoles dramatically reduces the proportion of good quality embryos reaching the blastocyst stage and negatively influence PR, implantation rates and early repeated pregnancy loss.

MSOME-ICSI is recommended according to specific indications such as different degrees of teratozoospermia, previous failure of implantation or absence of blastocyst formation in previous attempts.

MSOME may also be considered as a new approach for a sperm cytogram since vacuoles are more accurately observed as compared to Kruger criteria. IMSI can be offered to patients when a previous spermogram with MSOME assessment reveals a high percentage of vacuoles. However, at the present time there is no threshold of the percentage of normal forms for either recommending ICSI or IMSI.

The fundamental question is not under which conditions IMSI should be recommended but if MSOME should always be considered to select the best spermatozoa. Assessment of sperm morphology by Kruger's strict criteria is routinely used and widely accepted as the best predictor of male fertility potential, better than sperm concentration or motility, highlighting the concept that sperm morphology is the most important parameter in the semen analysis. As consequence, we have to ask ourselves if there are still indications where improved sperm-selection before fertilization is not necessary or low-magnification microscopy using Hoffman modulation contrast is more than enough? Probably not.

However, there are some limiting factors, such as the severity of either the teratozoospermia or oligozoospermia that render MSOME selection not possible.

IMSI is now a reality in ART practise but still with a lot of question marks regarding: (i) the terminology of vacuoles, their classification, their location on the sperm head, their origin and meaning, (ii) the application of IMSI instead of IVF in cases of unexplained infertility, (iii) the age of the woman; is IMSI necessary in younger women with good quality oocytes where cytoplasmic factors might be able to repair sperm nuclear defects? (iii) the technical aspect. We have to be aware that this technique is demanding and has to be performed in the best working conditions not to impair the oocyte quality.

The introduction of IMSI has the advantage that embryologists realize that more attention has to be paid during sperm selection even in case of classical ICSI.

The application of IMSI leads to more and better quality blastocysts and as consequence it increases the chance to select the proper embryo for transfer with high implantation potential. Furthermore, the effect of using MSOME approach to select spermatozoa manifests itself when it is performed in combination with day 5 embryo culture of all fertilized oocytes.

SELECTED ORAL COMMUNICATION SESSION
SESSION 40: OVARIAN STIMULATION 2
Tuesday 29 June 2010  15:15 - 16:30

O-146  The transcriptome of corresponding cumulus and granulosa cells obtained in connection with ART
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Introduction: Throughout folliculogenesis granulosa cells differentiate and proliferate from a total of 30-40 cells in the primordial follicle to around 50 millions in the pre-ovulatory follicle: numerous mural granulosa cells and around 1000 cumulus cells immediately surrounding the oocyte. It has been established that the oocyte governs this differentiation into the two somatic cell compartments in order to undergo an optimal growth and maturation. In the final stages of the folliculogenesis, cumulus cells maintain a close communication with the oocyte and take care of the milieu in its close proximity, whereas the granulosa cells ensure the optimal intra-follicular composition as well as the endocrine profile. Especially on a molecular level there is a paucity of information on which changes that characterizes the differentiation of cumulus and granulosa cells. Performing microarray analysis and focusing on the differences in gene expression will provide insight into the specialized function of the two cell types.

The aim of the present study was to compare the gene expression profile of corresponding cumulus and granulosa cells isolated from individual pre-ovulatory follicles immediately after ovum pick-up.

Materials and Methods: Granulosa and cumulus cells were obtained from one large (mean: 2.5ml) pre-ovulatory follicle from each of 12 women (mean age: 33 years) receiving controlled ovarian stimulation following a standard antagonist protocol using recombinant FSH (Puregon®, Merck-Serono). The protocol was approved by the Danish Scientific-Ethical Committee (Ethical ApprovalNumber: FK 299017).

Within 20 minutes following ovocyte retrieval the granulosa and cumulus cells were isolated and rinsed in PBS (Mg + + and Ca + + free, 0.1 M PVA) with RNase inhibitor (Protector Rsne Inhibitor, Roche Diagnostic) and transferred to an 0.2 ml tube (MicroAmp, Applied Biosystems), flash frozen in liquid nitrogen and stored at -80°C until RNA extraction. Only cells from follicles containing MII oocytes were used in the study.

RNA was extracted by PicoPureTM Isolation Kit (Arcturus) and labelled with RNase inhibitor (Protector Rnase Inhibitor, Roche Diagnostic) and transferred to an 0.2 ml tube (MicroAmp, Applied Biosystems), flash frozen in liquid nitrogen and stored at -80°C until RNA extraction. Only cells from follicles containing MII oocytes were used in the study.

RNA was extracted by PicoPureTM Isolation Kit (Arcturus) and labelled according to the standard double amplification protocol and hybridized on the Human Genome U133 2.0 Plus array from Affymetrix (Affymetrix Inc.). The two cell types were analyzed and genes were defined as being differentially expressed if they were selected in the paired (within individual follicle) t-test
with Benjamini Hochberg multiple testing corrections with p-value below 0.01, fold-change larger than 2 and difference of means larger than 50.

Results: Based on these straight criteria, a relative high number of 1542 unique genes (representing 10% of the genes expressed) were differentially expressed in the two cell types. Biological function analysis revealed 43 gene ontology terms represented in the 1542 differentially expressed genes (p < 0.001) and functions as ‘lipid metabolic process’, ‘glycosaminoglycan metabolic’ and ‘mitochondria function’ were highly represented. Genes exclusively present in cumulus cells represent functions as ‘cell-communication’, ‘extra-cellular matrix’, ‘ion-channel activity’ and ‘signal-transduction’, while ‘lipid metabolism’, ‘vacuoles’ and ‘mitochondria function’ dominated in the group of genes expressed exclusively in the granulosa cells. Pointing out a few of the genes differentially expressed: Androgen receptor, CYP19A, calcium channel (CACNA1C), aquaporins (2 + 3), ACE2 were higher and Anti Müllerian Hormone exclusively expressed, whereas genes for Follicistatin, Inhibin A, CYP11A1, and estrogen, growth hormone and progesterone receptors were lower expressed in cumulus cells as compared to granulosa cells. In addition, correlations between gene expression levels of specific genes will be presented and their relevance for embryo quality discussed.

Conclusions: The transcriptome of the corresponding cumulus and granulosa cells surrounding MI oocytes differs significantly. Functional analyses of expressed and differentially expressed genes confirmed known and generated new insight into the biology of the preovulatory follicle. Several new transcripts not previously related to follicular biology were identified.

O-148 Dopa receptor 2 (drd2) activation inhibits VEGF secretion in granulosa luteinized cells: implications for OHSS treatment in low drd2 milieus

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Objective: A hallmark of ovarian hyperstimulation syndrome (OHSS) is hCG-induced ovarian vascular endothelial growth factor (VEGF) overexpression and secretion which increases vascular permeability (VP) after binding to VEGFR-2 (VEGFR2) on endothelial cells (EC). Dopamine receptor-2 (drd2) agonists reduce OHSS onset in humans by decreasing ovarian VP probably by inhibition of VEGFR2 activation (phosphorylation of VEGFR2 tyrosine sites) in an autocrine fashion in ovarian EC. However, preliminary data showed that drd2 is not expressed on ovarian EC. In this work we aimed to explore: 1) which alternative molecular mechanism of action might explain the efficacy of drd2 agonists in inhibiting OHSS in humans; and 2) whether differences in ovarian drd2 expression might explain higher sensitivity to OHSS onset in women with PCOS.

Material and Methods: In vitro studies were performed with granulosa cells (GC) isolated from normal responder (10-15 oocytes, E2 < 1500) women (N = 5) undergoing controlled ovarian stimulation at pick-up. Time-course and dose-response experiments were performed to explore the ability of hCG (0, 1, 5, 10, 50 and 100 IU) and drd2 agonist Cabergoline (Cb2; 0, 1, 5, 10, 100, 250 μM) to respectively increase and inhibit VEGF, E2 and P4 secretion in culture as determined by ELISA and EIA. Subsequently GC were isolated from other normal responders (N = 8), allowed to shed for 24 hrs, and further cultured for 72 hrs in the presence/absence of hCG (50u) and Cb2 (133 μM) in 96 well plates (10,000 cells/well). VEGF was quantified in culture and an IncF ELISA kit was used to quantify drd2 and VEGFR2 expression, and activation of VEGFR2 at phosphorylated Tyr951, Tyr996 and Tyr1054/1059 sites in seeded cells.

In a second set of experiments PCOS (N = 39) and non-PCOS(N = 51) human ovarian sample slides from our Pathology archives where employed to characterize and quantify drd2 expression during follicular and (early, mid) luteal and phase using immunohistochemistry and image pro-plus software analysis.

Results: The initial studies showed that the best response in VEGF and P4 secretion of GC was obtained after 4 days in culture and 133 μM Cb2, which was twice the dose able to induce an EDSO decrease in VEGF. GC showed a mean 3.9 fold reduction in VEGF secretion in each patient when Cb2 (433±277.3 pg/ml) compared to vehicle (1712.1±349.7 pg/ml, p < 0.01) was added to the culture medium. These effects were accompanied by a 37.3% and 56.7 % reduction in VEGFR2 and VEGFR2′ activation and induced 2 fold upregulation of drd2 in Cb2-treated cells.

Immunohistochemistry revealed the strongest drd2 signaling in GC and theca cells. A 2.7 and 4.2 reduced drd2 stained area was respectively observed for theca layer and GC in PCOS (3.7 ± 0.9%, 5.4 ± 1.7%) vs control (14.5 ± 2.3%, p < 0.01; 15.2 ± 4.2%, p < 0.05) samples. No staining for drd2 was found in ovarian EC in any sample.

Conclusions: While these data do not exclude that coexpression of drd2 and VEGFR2 might mediate “autocrine” mechanisms to control VP in other cells, lack of staining for drd2 in ovarian EC makes this mechanism of action very unlikely. Our data suggest a newly discovered mechanism where drd2 agonist act on GC inhibiting the secretion of VEGF, and therefore reducing the amount of ligand able to activate VEGFR2 explaining the reduced ovarian permeability observed in Cb2-treated subjects. In addition, lower presence of VEGFR2 and upregulation...
of drd2 induced by Cb2 in GC may also account for diminished VEGF signaling in a negative feedback loop. In such scenario, decreased drd2 expression, as observed in PCOS women, might lead to increased ovarian VEGF and VEGFR2 expression accounting for a higher risk of OHSS. Nevertheless we suggest drd2 agonists might still prevent OHSS in PCO women by upregulating drd2.

O-149 Intravenous fluids for prevention of severe ovarian hyperstimulation syndrome. Cochrane systematic review and meta-analysis

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Introduction: Ovarian hyperstimulation syndrome is serious and potentially fatal complication of ovarian stimulation which affects 1-14% of all IVF/ICSI cycles. A number of clinical studies with conflicting results have been reported in which intravenous fluids as albumin; hydroxyethyl starch, Haemaccel and dextrans have been tested as a possible way for preventing the severe form of OHSS. Our aim is to review the effectiveness and safety of administration of intravenous fluids in prevention of severe ovarian hyperstimulation syndrome in IVF/ICSI treatment cycles.

Material and Methods: We searched the Cochrane Menstrual Disorders and Subfertility Group, Specialised Register of controlled trials, the Cochrane Central Register of Controlled trials (up 3rd Quarter 2009), MEDLINE (1950 up to Sept.2009), EMBASE (1980 up to Sept.2009) and The National Research Register (up to September 2009). The citation lists of relevant publications, review articles, abstracts of scientific meetings and included studies were also searched. The authors were contacted to identify or clarify data that were unclear from the trial reports. Selection criteria: Randomised controlled trials which compared the effect of intravenous fluids with placebo or no treatment for the prevention of severe OHSS in high risk women undergoing IVF/ICSI treatment cycles. Data collection and analysis: Two review authors independently scanned the abstracts, identified relevant papers, assessed inclusion and trial quality and extracted relevant data. Validity was assessed in terms of method of randomisation, allocation concealment and outcomes. Where possible, data were pooled for analysis. A separate analysis of studies used human albumin and hydroxyethyl starch versus placebo or no treatment was performed. Other potential intravenous fluids have also been identified such as haemaccel and dextran, however, no randomised controlled studies on their applicability could be found. Primary outcome measure was severe ovarian hyperstimulation syndrome incidence per randomised women. Secondary outcome measures were live birth rate, ongoing pregnancy rate, clinical pregnancy rate, and miscarriage rate. Searches (until Sep 2009) were conducted in MEDLINE, EMBASE, Science Direct, Cochrane Library and databases of abstracts.

Data collection and analysis: Two review authors independently scanned the abstracts, identified relevant papers, assessed inclusion and trial quality and extracted relevant data. Validity was assessed in terms of method of randomisation, allocation concealment and outcomes. Primary outcome measure was OHSS incidence per randomised woman. Secondary outcome measures were Live birth rate clinical pregnancy rate, early miscarriage rate.

Results: Four randomized trials entailing 570 women were included. There was evidence of statistically significant reduction in the incidence of OHSS in the dopamine agonist group (OR 0.41 95% CI 0.25, to 0.66) with an absolute risk reduction of 12 % (95% CI 6.07% to 18.2 %). There was no evidence of a reduction in severe OHSS (OR: 0.50, 95% CI 0.20 to 1.26). There was no evidence for a difference in clinical pregnancy rate (OR: 1.07, 95% CI (0.70 to 1.62) and miscarriage rate (OR: 0.31, 95% CI (0.03 to 3.07). Conclusion: We conclude that dopamine agonist as a preventive treatment leads to a significantly lower OHSS incidence in high risk patients without compromising pregnancy outcomes.

SELECTED ORAL COMMUNICATION SESSION

SESSION 41: EMBRYO DEVELOPMENT & IMPLANTATION

Tuesday 29 June 2010 15:15 - 16:30

O-151 Embryos with too early first cleavage fail to implant; correlation between exact timing of first cleavage and successful implantation

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Introduction: Accurate assessment of embryo quality is particularly important in IVF treatments to achieve a successful pregnancy. The use of an automated instrument with programmable time-lapse image acquisition, allows data to be collected for individual embryos during development to quantify the timing of each cell division. Our objective was to validate the EmbryoScope time-lapse system for determining the exact timing of the first cleavage in order to characterize those embryos which successfully implant.

Material and Methods: Development of 1643 embryos from 158 couples undergoing first ICSI cycles were analyzed by using the EmbryoScope monitoring system (Unisense Fertilitech, Aarhus Denmark). The EmbryoScope is comprised of a tri-gas incubator with a built-in camera which acquires images at multiple focal planes at preset time intervals (e.g. every 15 to 20 minutes). A time-lapse film of the embryos is generated which allows analysis of onset and duration of cell divisions. 158 treatment cycles with transfer of embryos after 3 day incubation in the EmbryoScope gave rise to 93 pregnancies (59%). We selected 82 transferred embryos for detailed analysis based on either: 100%