determine access to IVF. The object of the study is to share that experience from the perspective of the Chair of the PRP.

Results: The range and nature of applications determined by the PRP will be considered. These include presumptions against treatment because of criminal records and involvement with child protection services or risk of abuse or neglect; extended storage of gametes and embryos; approval of surrogacy arrangements; posthumous use of gametes and embryos and PGD for sex selection. The results of appeals from decisions of the PRP and the reasons therefore will be outlined.

Conclusions: Issues arising from the legislative scheme for the PRP and for IVF clinics and patients will be addressed.

Special attention will be given to such contentious legal, moral and ethical issues as commercial and international surrogacy and sex selection

P-268 Effects of the healthcare modernisation law on infertility treatment outcomes in Germany: a retrospective cohort study

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Introduction: Since 2004 infertile couples in Germany face a 50% co-payment of assisted reproduction techniques for the first three treatments as part of the healthcare modernisation law. Aim of this retrospective cohort study is to examine the effects of this law on outcomes of infertility treatment by studying data of the German IVF Register (DIR) between 2000 and 2007.

Material and Methods: 586058 treatment cycles and a total of 357437 women that underwent infertility treatment in the fertility centres participating in DIR between 2000 and 2007 were utilized in this study. The data were published in the DIR annual reports between 2000 and 2007. The treatment rate, birth rate, rate of new born children after infertility treatment of German women between 25 and 39 years, pregnancy rate, baby take home rate, twin pregnancy rate and mean transferred embryos per cycle were examined. The relative risk was estimated for all parameters between the two compared groups of the years 2000-2002 and 2004-2007. Data of the years 2003 and 2004 were excluded, so that short-term effects before and after intervention could be minimized.

Results: While the treatment rate was reduced to 5.1%, the rate of women with additional treatment in the same year was reduced to 2.1%. Apart from that, the birth rate after infertility treatment was reduced to 8.1%, while the new born children rate was reduced to 9.1% between the two compared groups. The pregnancy rate a baby take home rate after fresh embryo transfer were increased to 7.0% and 14.4%, respectively. All results were statistically significant. The twin pregnancy rate was significantly reduced to 8.2%. Mean transferred embryos per cycle were reduced from 2.29 in 2000 to 2.07 in 2007.

Conclusions: Healthcare modernisation law led to a short-term reduction of treatment rates, birth rates, twin pregnancy rate, mean transferred embryos per cycle. The result was further analysed by real-time-PCR. Proliferation marker MKI67 and apoptosis marker TP53 were analysed along with apoptotic index by TUNEL assay. Ethical permission for this study was obtained prior to start of the study.

Results: Twenty one canonical pathways showed significantly different expression (p < 0.05) on comparing between good and poor responders. The most differently expressed pathway was Metabolism of Xenobiotics by Cytochrome P450 pathway. The second most significant pathway and the pathway more relevant to uterine leiomyoma growth is the glutathione pathway harboring glutathione-s transferases (p = 0.0001, ratio 5%). One of the genes was downregulated (GPX2 – 1.7 fold) and 4 genes belonging to this family were upregulated (GSTM1 + 8.0-fold), GSTM2(+ 1.5 fold), GSTM3(+ 2.3 fold), GSTM5(+ 2.2 fold) among the good responders. Further analysis by real time PCR showed GSTM1 was not detectable in biopsies from non responders. No correlations were seen for GSTM1, MKI67 or TP53 versus percentual myoma volume reduction. TUNEL analysis showed no difference in the degree of apoptosis between good or bad responders to mifepristone.

Conclusion: Our findings indicate that glutathione pathway is involved in the action of mifepristone on leiomyoma volume reduction. GSTM1 positive phenotype is of importance for uterine leiomyoma volume reduction in response to mifepristone exposure in vivo. The mechanism behind the difference in growthregulation is still not clear, but could be suggested to interfere with proliferation or repression co-regulators related to the degree of metabolism of steroids regulated by GSTMs. The finding in the present study of a tentative prognostic marker for leiomyoma volume reduction during mifepristone treatment is of potential importance for the clinical management of millions of women suffering from symptoms from uterine leiomyomas.

P-270 Aminopeptidase activity in human follicular fluid

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Introduction: Enkephalins (ENK) were the first endogenous opioid peptides isolated from brain tissue and sequenced (1975). To date, these substances are implicated in a wide range of physiological processes, with their roles in behaviour, neuroendocrinology and pain transmission being the best documented. However, their function in female reproduction is completely unknown and controversial in some aspects.

POSTER VIEWING SESSION

FEMALE (IN) FERTILITY

P-269 Glutathione pathway gene expression determines good or poor response to mifepristone in myoma volume regression

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Aim: To explore the molecular basis of clinically observed volume reduction in uterine leiomyomas exposed to mifepristone.

Background: Treatment of uterine leiomyomas with a selective progesterone receptor modulator (SPRM) as an alternative to surgery is of considerable clinical interest. Steroid hormone receptors are overexpressed in leiomyoma tumor tissue compared to adjacent myometrium and involved in the process of leiomyoma growth. Progesterone receptor modulators such as mifepristone are effective and well tolerated in reducing myoma volume and vaginal bleeding. In a previously reported study we observed a significant volume reduction in the dominant myoma in response to mifepristone, but with a wide individual variation (median -23%, range: -81 to +19%) in response to treatment. Thus, a study was conducted to explore the molecular basis of good response to mifepristone treatment.

Material and Methods: Premenopausal women with uterine leiomyoma (n = 12) received treatment with mifepristone 50 mg every other day for 12 weeks. Among them, eight women were sub grouped as good (N = 4, median –49%, range –64 to –31%) or poor (N = 4, median –22%, range –23 to –21%) responders. At surgery, biopsies were taken from the periphery of the dominant leiomyoma and total RNA was extracted to study the gene expression by microarray. The result was further analysed by Ingenuity Pathway Analysis (IPA, Ingenuity® Systems, www.ingenuity.com) to explore the leading molecular pathway mediating the response to mifepristone. The result from the microarray was confirmed by real time-PCR. Proliferation marker MKI67 and apoptosis marker TP53 were analysed along with apoptotic index by TUNEL assay. Ethical permission for this study was obtained prior to start of the study.

Results: While the treatment rate was reduced to 5.1%, the rate of women with additional treatment in the same year was reduced to 2.1%. Apart from that, the birth rate after infertility treatment was reduced to 8.1%, while the new born children rate was reduced to 9.1% between the two compared groups. The pregnancy rate a baby take home rate after fresh embryo transfer were increased to 7.0% and 14.4%, respectively. All results were statistically significant. The twin pregnancy rate was significantly reduced to 8.2%. Mean transferred embryos per cycle were reduced from 2.29 in 2000 to 2.07 in 2007.

Conclusions: Healthcare modernisation law led to a short-term reduction of treatment cycles, oocyte retrievals as well as deliveries and newborn children rate was reduced to 9.1% between the two compared groups. The pregnancy rate a baby take home rate after fresh embryo transfer were increased to 7.0% and 14.4%, respectively. All results were statistically significant. The twin pregnancy rate was significantly reduced to 8.2%. Mean transferred embryos per cycle were reduced from 2.29 in 2000 to 2.07 in 2007.
The effects of peptides can be regulated by their enzymatic hydrolysis. ENKs are particularly susceptible to hydrolysis by peptidases due to their short chains. One enzymatic pathway considered of great importance in the degradation of ENKs is the break-down of the Tyr-Gly bond by aminopeptidases. Among this group it is important to highlight the activity of three enzymes: the aminopeptidase N (APN; EC 3.4.11.2), the puromycin-sensitive alanyl aminopeptidase (PSA; EC 3.4.11.14) and the aminopeptidase B (APB; EC 3.4.11.6).

The ovarian follicular fluid (FF) represents a complex functional compartment that integrates endocrine, immunological and mitogenic signals which makes it unique. Granulosa cells produce substances that may be worth in FF setting different parameters of quality. In fact, it is the first time that the presence of APN, PSA and APB has been described in FF. Furthermore, while the activity of APN and PSA is quite similar, the activity of APB is statistically lower. Nevertheless, the function of enkephalin metabolism in FF is still far from understood.

The aim of the present study was to measure the activity of the enkephalin-degrading enzymes; APN, PSA and APB in follicular fluid, and to evaluate their possible involvement in female reproductive pathologies. To this end, we examined and compared the level of activity from fertile woman (group FERT) and in patients with pathologies such as polycystic ovarian syndrome (group PCOS), ovarian endometriosis (group END), tubal factor (group TF) and unexplained infertility (group UI).

Material and Methods: At the oocyte retrieval FF was collected from the first follicle aspirated, fluid should be clear and not contaminated with blood. FERT’s age was 24.86 ± 3 years (mean ± S) (n = 24), PCOS (n = 8), END (n = 10), TF (n = 15) and UI (n = 12). In every group APN, PSA and APB activities were fluorometrically measured using β-nafthalilamida as substrate. The assay is based on the fluorescence of β-nafthalilamina generated from the hydrolysis of the substrate by the enzyme. The excitation and emission wave lengths were 345 nm and 412 nm respectively. In all cases, one unit of enzyme activity is considered as the amount of enzyme that hydrolyzes 1 pmol of substrate per minute, the entire activities are expressed as units of peptidase activity per litre of sample.

Results: APN, PSA and APB activity was detected in all FF samples. The aminopeptidase activity of the enkephalin-degrading enzymes; APN, PSA and APB in follicular fluid, and to evaluate their possible involvement in female reproductive pathologies. APN and PSA have been described in FF. Furthermore, while the activity of APN and PSA is quite similar, the activity of APB is statistically lower. Nevertheless, the function of enkephalin metabolism in FF is still far from understood.

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The role of the postcoital test (PCT) is heavily debated. Yet, tozoon per high power field (400x). Couples were treated according to the work-up.

Introduction: The aim of the study was to determine the effect of a treatment with myo-inositol (MI) plus folic acid (FA) plus melatonin (M) compared with myo-inositol plus folic acid alone on oocyte quality in polycystic ovarian syndrome (PCOS) women underwent in vitro fertilization (IVF) cycles.

Material and Methods: 112 women were enrolled in the study and divided into two groups: group A (56 patients) received MI + FA 2 g and group B (56 patients) received MI + FA 2 g + M, administered continuously twicew a day. All women underwent fixed antagonist protocol. Primary endpoints were number of morphologically mature oocytes retrieved (MII oocytes), embryo quality, and pregnancy and implantation rates. Secondary endpoints were the total number of days of FSH stimulation, total dose of gonadotropin administered, E2 level on the day of triggering, the total number of oocytes retrieved (immature and mature oocytes), fertilization rate per number of retrieved oocytes and embryo cleavage rate, live birth and miscarriage rates, cancellation rate and incidence of moderate or severe ovarian hyperstimulation syndrome.

Results: Total r-FSH units (1,384 ± 675 vs 2,288 ± 590) and number of days of stimulation (11.6 ± 0.9 vs 13.1 ± 1.3) and peak E2 levels (2,243 ± 576 vs 2,843 ± 595 pg/ml) at hCG administration were were significantly reduced in patients receiving myo-inositol + melatonin. The mean number of oocytes retrieved did not differ between the two groups (7.89 ± 1.72 vs 7.47 ± 1.78; P = 0.65), whereas in the group cotreated with melatonin the mean number of germinal vesicles and dysmorphic oocytes was significantly reduced (1.0 ± 0.9 vs 1.6 ± 1.0), with a trend for increased percentage of oocytes in metaphase II (6.88 ± 1.67 vs 5.46 ± 1.59; P = 0.047) and a lower mean number of immature oocytes (2.31 ± 0.84 vs 2.28 ± 0.75; P = 0.001). The mean number of embryos of top-quality (class 1 and 2) resulted higher in the group A (3.2 ± 0.64 vs 2.28 ± 0.75; P = 0.01). Fertilization rate did not differ between the two groups. A total of 36 pregnancies were obtained (21 in group A and 15 in group B; P = 0.26). Clinical pregnancy rate and implantation rate were in tendency higher in the group cotreated with melatonin, although the differences did not reach statistical significance. Biochemical pregnancy rate and abortion rate were similar in both groups.

Conclusion: These data show that in PCOS patients, treatment with myo-inositol and melatonin, but not myo-inositol alone, reduce germinal vesicles and dysmorphic oocytes at pick-up without compromising total number of retrieved oocytes. This approach reducing E2 levels at hCG administration, could be adopted to decrease the risk of hyperstimulation in such patients. Melatonin ameliorates the activity of myo-inositol and folic acid by improving oocyte quality and pregnancy outcome in women with low oocyte quality history.

P-274 Overall ongoing pregnancy rate and mode of conception after a positive and a negative postcoital test in the fertility work-up

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Introduction: The role of the postcoital test (PCT) is heavily debated. Yet, it is a significant factor in prognostic models that predict spontaneous pregnancy rate within one year. Information on the long-term outcome and the contribution of treatments like IUI and IVF on the overall pregnancy rate is lacking. The aim of this study was to evaluate the long-term outcome of subfertile couples with a positive and a negative PCT during their fertility work-up.

Material and Methods: In a longitudinal, multicentre observational cohort study 1613 couples underwent a PCT as part of their work-up. Couples with anovulation, tubal disease, endometriosis and severe male factor infertility were excluded, leaving 933 couples for analysis. All PCTs were timed by ultrasound. A positive PCT was defined as at least one propulsive spermatozoon per high power field (400x). Couples were treated according to the standing national treatment protocols. Possible treatment options were, subsequently, expectant management, IUI (intrauterine insemination) or ART (i.e. IVF or ICSI). Primary outcome measures were spontaneous ongoing pregnancy rate and overall ongoing pregnancy rate during a follow-up of 5 years. Secondary outcome measures were the number of couples that had to undergo IUI and IVF and the contribution of these treatments to the overall ongoing pregnancy rate.

Results: 60.1% of the couples had a positive PCT, 39.9% a negative PCT. The spontaneous ongoing pregnancy after 5 years was 55.1% and 30.4%, respectively (p < 0.001). The overall pregnancy rate was 78.1% and 70.9%, respectively (p < 0.02). The contribution of IUI to the ongoing pregnancies was 11.1% and 18.9%, respectively (p < 0.01) and the contribution of ART 11.9% and 22.6% (p < 0.001). The proportion of couples that underwent IUI treatment after a positive PCT was 39.9%, after a negative test 46.5% (p < 0.03). The proportion of couples that underwent ART was 20.9% and 37.4%, respectively (p < 0.001).

Conclusions: Couples with a negative PCT have a significantly lower spontaneous ongoing pregnancy rate after 5 years of follow-up compared to couples with a positive PCT. Also the overall ongoing pregnancy after 5 years is significantly lower in the couples with a negative PCT, despite a larger use of IUI and IVF. Our data do not support the trend to abolish the PCT from the fertility work-up.

P-275 Can three dimensional transvaginal ultrasonography replace hysterosalpingography in diagnosing uterine anomalies?

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Introduction: Hysterosalpingography (HSG) is a frequently performed procedure, that plays an important role in evaluating the uterus and fallopian tubes. The most typical indication for HSG is infertility and recurrent miscarriage, which are common problems in the female population. However, this procedure is invasive, exposes the patient to ionizing radiation and carries a risk of complications. Other imaging modalities include ultrasound examination and magnetic resonance imaging (MRI).

MRI is the most accurate technique for visualizing the female genitourinary tract anatomy, but, as it is expensive and not readily available, it is not useful in everyday practice.

Three dimensional transvaginal ultrasound (3D TV USG) is a noninvasive and quick, imaging method that in some cases may replace HSG.

Material and Methods: In this study we compared the HSG and 3D TV USG diagnoses obtained in 50 patients referred to our Department because of infertility and/or suspected uterine anomalies. The studied group consisted of 42 patients with normal uterus, 2 with arcuate, 1 with unicornuate, 2 with septate and 3 with didelphys uterus.

Results: In all of the cases, HSG diagnoses were consistent with 3D TV USG findings. All uterine anomalies were then confirmed by hysteroscopy and/or laparoscopy.

Conclusions: 3D TV USG can replace HSG in imaging of uterine anomalies, especially in cases where the assessment of tubal patency is not necessary.

P-276 Sonographic parameters in assessing endometrial receptivity in infertile smoking and non smoking women

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Introduction: Vascularization of the female reproductive system seems to play an important role in the uterine receptivity. In the uterus, angiogenesis is essential for endometrial growth and maturation. Endometrial blood flow may predict the success of conception. Cigarette smoking affects female fertility, reduces uterine receptivity and inhibits endometrial cell proliferation through a nitric oxide-mediated pathway.

Objective: The aim of this study was to investigate the role of subendometrial blood flow in unexplained infertility in smoking and nonsmoking women. All
patients were less than 35 years old, had body mass indices between 19 and 30 and serum FSH levels below 10 IU/l in the early proliferative phase.

**Material and Methods:** Prospective study of 22 smoking women with unexplained infertility, 25 nonsmoking women with unexplained infertility and 20 fertile nonsmoking controls. The mean spiral artery pulsatility index values, resistance index values and peak systolic velocity were recorded. The area of interest was defined as the subendometrial area within 5 mm of the endometrial borders in the midluteal phase of the menstrual cycle.

**Results:**

<table>
<thead>
<tr>
<th>Smoking infertile</th>
<th>Nonsmoking infertile</th>
<th>Nonsmoking fertile</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral artery PI</td>
<td>1.52 (1.05-1.64)</td>
<td>1.28 (1.02-1.35)</td>
<td>1.10 (0.88-1.23)</td>
</tr>
<tr>
<td>Spiral artery RI</td>
<td>0.91 (0.84-0.99)</td>
<td>0.86 (0.77-0.96)</td>
<td>0.81 (0.67-0.85)</td>
</tr>
<tr>
<td>Spiral artery PSV</td>
<td>18.13 (15.10-21.34)</td>
<td>14.32 (12.51-17.12)</td>
<td>10.57 (9.21-12.24)</td>
</tr>
</tbody>
</table>

*Statistically significant differences between smoking infertile and nonsmoking fertile groups (ANOVA).

Mean spiral artery pulsatility index values and peak systolic velocity were significantly increased in smoking infertile women in comparison to the control group, resistance index values remained unchanged. In nonsmoking infertile women subendometrial blood flow parameters were not significantly different from the control group.

**Conclusion:** Subendometrial blood flow is significantly reduced in cigarette smoking infertile women.

**P-277 Expression and localization of opioid receptors in human immature oocytes and in unfertilized metaphase II oocytes**

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**Introduction:** Endogenous opioid peptides and opioid receptors have been characterized in some of the female reproductive system organs, but little is known about the expression of these receptors in human oocytes and much less about the potential functions of opioids in oocyte maturation. The aim of the study was to describe the expression of opioid receptors in human oocytes and to investigate the differential distribution of three opioid receptors (DOR, KOR and MOR) at various stages of meiotic resumption in human oocytes.

**Methods:** A total of 604 human oocytes from 191 patients (aged 25–39) undergoing intracytoplasmic sperm injection (ICSI), were analyzed using Western blot, immunocytochemistry and PCR techniques. The oocyte maturation stages were distributed as follows: 154 were at the germinal vesicle (GV) stage, 102 at metaphase I (MI) and 348 at metaphase II (MII).

**Results:** Western blot analysis revealed the presence of the Delta (DOR), Kappa (KOR) and Mu (MOR) opioid receptor proteins in human oocytes. The MOR and KOR immunostaining patterns changed during the various stages of meiotic resumption, while the DOR pattern was the same throughout.

**Conclusion:** We report for the first time that opioid receptors DOR, KOR and MOR are present in human oocytes and that their localization change in the different stages of meiotic resumption. This fact suggests a possible action of the opioids via receptors in the maturation of the gamete and maybe in the process of fertilization.

**P-278 Pigment epithelium derived factor (PEDF), a crucial regulator of ovarian**

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Abstract withdrawn by the author

**P-279 Immobilisation versus immediate mobilisation after intrauterine insemination: long term follow up**

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**Introduction:** A previous randomized clinical trial comparing immediate mobilisation after intrauterine insemination (IUI) and fifteen minutes immobilisation after IUI, showed after three cycles a significantly higher number of live births in the group immobilised for fifteen minutes (27% vs 18%). In the present study, we investigated the impact of this difference on the three year pregnancy rates in both groups.

**Material and Methods:** In the original study 391 subfertile couples were eligible for IUI were randomized between three cycles of IUI with immediate immobilisation (192 couples) or three cycles of IUI with fifteen minutes immobilisation afterwards (199 couples). After these cycles couples received treatment according to local protocol. In most cases this included more IUI cycles, with or without controlled ovarian hyperstimulation, followed by in vitro fertilisation. Couples were followed until three years after randomisation and pregnancies and treatments were registered. Primary endpoint was an ongoing pregnancy, defined by fetal heartbeat seen by transvaginal ultrasonography at 12 weeks’ of gestation. Secondary endpoints were live births, miscarriages, ectopic pregnancies, multiple pregnancies, the time to pregnancy and applied treatment. Analysis was by intention to treat. A two-tailed Fisher’s exact test was used to test for significance. Time to pregnancy was evaluated with a Kaplan Meier curve and a log rank test.

**Results:** The number of ongoing pregnancies after three years was significantly higher in the immobilisation group: 137 (69%) versus 108 (56%), P = 0.012 (RR 1.2 (95% CI 1.0 – 1.4)).

**Conclusion:** The increase in pregnancy rates that was observed in women receiving IUI with 15 minutes of immobilisation compared to IUI with immediate immobilisation persists even after three years.

**Reference:**

Introduction: It is known from studies in animals and human that follicle size is related to oocyte developmental competence. Recently gene expression analysis in cumulus complexes (CC) demonstrated a variance between the different cumulus oocyte complexes (COC) from each patient and these within patient expression variances were related to oocyte competence.

The variability in CC gene expression within a patient might thus be dependent on follicle size. In this study, follicle size, measured as the follicle fluid volume aspirated at oocyte retrieval, was related to CC gene expression of 8 oocyte quality genes. The aim of the study was to set up a multiple regression model, allowing for correction of between-patient variances, considering patient-dependent and stimulation-dependent variables.

Material and Methods: From 17 ICSI patients (7 GnRH agonist/HP-metrotropin and 10 GnRH antagonist/recombinant FSH) follicular fluid volume was recorded and related to 85 cumulus oocyte complexes (COC) enclosing MII oocytes. CC were collected from all COCs and were handled individually from the moment of pick-up on. Two groups of variables were recorded. A first group comprised the patient related factors: age, BMI, ovarian hyperstimulation protocol, ovarian responsiveness (number of COCs at pick up/daily gonadotrophin doses) and serum levels of LH, FSH, estradiol and progesterone on day of hCG. A second group, oocyte-specific variables, contained the embryology data on day3: number of cells, fragmentation rate and an overall embryo quality score (1 to 4).

The cumulus cells of 40 oocytes (from 4 agonist/HP-hMG and 4 antagonist/rFSH stimulated patients) were analysed by real-time PCR for activated leukocyte cell adhesion molecule (ALCAM), prostaglandin-endoperoxide synthase 2 (PTGS2), gremlin 1 (GREM1), versican (VCAN), syndecan 4 (SDC4), transient receptor potential cation channel, subfamily M, member 7 (TRPM7), calmodulin 2 (CALM2) and inositol 1,4,5-triphosphate 3-kinase A (ITPKA).

Of 45 additional oocytes (3 agonist HP-hMG and 6 antagonist rFSH stimulated patients) CC were analysed for SDC4, TRPM7, CALM2 and ITPKA. All were normalized to UBC and B2M expression.

A stepwise regression model selection (with 3 forward and 1 backwards step) was applied to identify the three main variables related to gene expression levels using either oocyte related parameters and a general patient nominator or oocyte and patient-specific variables.

Results: A first screening with 8 genes (4 COCs) showed that CALM2, TRPM7, ITPKA (p < 0.05) and SDC4 (p > 0.05) retained the variable follicle fluid volume as determining factor of the gene expression level in a model including oocyte related parameters and a general patient factor correcting for between patient variation. Therefore the experiment was extended with extra patients for those genes (total 85 COCs).

For the 85 COC dataset, specific patient characteristics were also allowed into the models and for the four genes follicle volume was one of the three main parameters determining the differences in gene expression. For TRPM7, CALM2 and SDC4 the follicle volume was the only oocyte-specific variable retained next to two patient related variables, whereas ITPKA variance depended only on oocyte-specific variables.

The correlation between follicle volume and gene expression in CC was inverse and strongest for ITPKA (p < 0.05, model p < 0.01).

Conclusions: Follicle size could, at least partially, explain the within patient variation in expression in the cumulus complexes for four genes, but the correlations were not very strong. We therefore hypothesize that within patient expression variation might be more dependent on oocyte than on follicle size characteristics.

P-281  The role of acupuncture in ART: preliminary results of an ongoing prospective randomized study
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Introduction: Acupuncture is an ancient Chinese therapeutic art, which has been given renewed attention for the treatment or for the integration of traditional therapy of several diseases, including infertility. However, the clinical validity of acupuncture is still debated for the difficulty to conduct rigorous prospective randomized clinical trials.

Starting in September 2009, a study was designed in our Clinic to investigate the effect of acupuncture in the reproductive outcome of patients undergoing assisted reproductive technologies (ART).

Preliminary results are reported below.

Material and Methods: Couples admitted to in vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI) treatments are invited to take part in the prospective randomized study. The only exclusion criteria is age ≥ 40 yrs.

Patients who accept to enter the trial are randomized in two groups: group A receiving acupuncture (study group) and group B not receiving acupuncture (control group).

By design, acupuncture procedures have to be administered by the same professional practitioner. The strategy chosen includes three sessions lasting 25 minutes each aiming to positively influence oocyte maturation during controlled ovarian hyperstimulation (COH) and embryo implantation. The first session is planned in the mid follicular phase (5-7 days before egg retrieval) stimulating the following points: guanyuan (CV4); lieque (7LU); guaï (29ST); sanyinjiao (6SP); gongsun (4SP); shenmen 55 ear point. The second session is planned in the late follicular phase (2-3 days before the egg retrieval) stimulating the acupoints: guanyuan (CV4); guaï (29ST); zusani (36ST); sanyinjiao (6SP); taiiouchong (3LR); neiguan (6PC); shenmen 55 ear point. The third session is performed shortly after embryo transfer, stimulating the points: guanyuan (CV4); gaulaï (29ST); zusani (36ST); taiiouchong (3LR); neiguan (6PC); diï (8SP); shenmen 55 ear point. Acupuncture is performed using a 4-cm long disposable stainless steel needles.

Apart from acupuncture, patients in group A and B are similarly treated according to the procedures implemented at S.I.S.Me.R.

The study was approved by the Ethical Committee.

Results: Between September 2009 and November 2010, approximately 305 patients aged ≥40 yrs started their IVF/ICSI treatment in our IVF center. Once clearly informed on the study (purpose, time requested, etc), 46 women signed the consent form to undergo the study: 23 were randomized in group A and 23 in group B. Female age (35.1 vs 34.6 yrs) and indication to ART were similar in the two groups. Ovarian response: 2020 ± 1000 vs 2070 ± 900 IU of FSH were administered (ns); estradiol at human chorionic gonadotropin (HCG) was respectively 1209 ± 117 pg/ml and 1285 ± 180 pg/ml (ns); 7.4 ± 2.7 and 6.95 ± 2.9 mean oocytes collected (ns).

IVF/ICSI performance: 90.1 vs 77.6 fertilization rate (χ² 3.2 -ns); 92 vs 96 cleavage rate (ns). A similar mean number of embryos were transferred in the two group (1.92 vs 1.76); the PR/ET and the implantation rate resulted respectively 47 vs 45 (ns) and 27 vs 24 (ns). The live birth rate (or ongoing pregnancy PR > 22 weeks) per egg retrieval resulted exactly the same: 35% vs 35%.

Conclusions: Rigorous prospective trials involving repeated acupuncture sessions may require long time and continuous efforts to enroll and treat a consistent population of subjects. The limited number of patients included so far in the study does not permit to draw any conclusions. However, the preliminary results observed in our study do not support the hypothesis of a positive effect of acupuncture in the reproductive performance for patients undergoing IVF/ICSI cycles. The study will continue to reach higher statistical power in order to clarify the debate on this topic.

P-282  Prospective assessment of automated follicular monitoring by 3D ultrasound and SonoAVC software: clinical applicability of the BT9 version in routine IVF patients
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Introduction: Ovulation induction monitoring requires serial measurements of ovarian follicles as well as estradiol determinations. Automatic follicle tracking by 3D ultrasound (US) and SonoAVC (Automated Volume Calculation, GE medical systems, Zipf, Austria) has recently been introduced in clinical practice (Fertil Steril 2010;93:616) and has been used routinely in our IVF program since March 2008. However, the first version of the software allowed optimal image quality in only 60% of IVF patients, whereas in the remainder 40% image quality was not good enough to allow efficient automatic measurement of all follicles, limiting the clinical applicability of this technology. The present study was designed to assess image quality in IVF cycles using a newer version
P-284 Is leptin concentration in follicular fluid and blood a dependable predictor of In Vitro Fertilization success?

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Introduction: In the past decade several studies have attempted to establish the association between leptin levels in either the serum or follicular fluid and the outcome of IVF cycles in humans with contradictory results. Thus, the goal of this study was to examine the relationship between follicular fluid (FF) and serum leptin levels with estradiol (E2), follicle stimulating hormone (FSH) and IVF success rates.

Material and Methods: After obtaining informed consent, 72 women, underwent controlled ovarian stimulation protocols for IVF/ICSI procedure. None had a history of diabetes mellitus, polycystic ovary syndrome, endometriosis or any other endocrine disorder. Ten patients with day three basal FSH >10 mIU/mL were excluded. Oocytes were isolated and evaluated and FF and centrifuged to remove debris, after which it was aliquoted and stored at -70°C until assayed. Embryos were assessed daily until the time of embryo transfer. An initial serum sample was taken in the follicular phase on day 3 of the cycle before meals, while second was taken on the morning of oocyte pickup before the mid-day meal. The content of each follicle was collected separately in a single syringe.

Results: Quality of automated 3D imaging was considered good when all follicles were measured automatically and no or minimal post-processing work was needed; fair when >50% of follicles needed manual measurement in the 3D scan; poor when ≥50% of follicles needed manual measurement.

Results: Quality of automated 3D imaging using the BT9 version of SonoAVC was good in 91% (n = 91), fair in 7% (n = 7) and poor in 2% (n = 2) of cases. In patients with good image quality average time for completion of the study with automatic monitoring by SonoAVC was 5.6 minutes as compared to 9.6 for manual monitoring. In patients with fair and poor image quality the time necessary for the study, including the postprocessing work was greater than the time needed for manual measurements.

Conclusion: With the updated version of the SonoAVC software acquisition of automatic follicular measures has been significantly improved compared to the previous version, allowing the implementation of this new technology in the majority of IVF patients. In our hands at least 90% of IVF patients have good image quality and in these women a considerable amount of time is saved when SonoAVC is used compared to manual 2D US monitoring. Since several investigators so far have shown that automatic measurements are at least as reliable as manual measurements, we believe that automatic 3D US with SonoAVC should be the preferred method for patient monitoring in IVF.

P-284 A short GnRH antagonist stimulation protocol generates an unexplained variance in the cumulus cell gene expression of cumulus-oocyte complexes at pick up

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Introduction: Cumulus cell (CC) gene expression analysis is suggested as a new tool to identify the most competent oocytes in ART treatment. The expression level of several oocyte quality genes in the CC is also regulated by patient and cycle characteristics and by the gonadotrophin preparation used. CC gene expression patterns were used to compare the cumulus-oocyte complexes (COCs) obtained from GnRH agonist and antagonist treatment cycles.

Material and Methods: Fifty-four ICSI patients were stimulated with recombinant FSH after a long GnRH agonist (n = 26) or in a short GnRH antagonist (n = 28) protocol. Thirteen variables were recorded: Age, BMI, duration of stimulation, serum concentrations of FSH, LH, progesterone and 17β-estradiol on day of hCG administration and on the day of oocyte retrieval procedure, oocyte maturity, number of oocytes, and three embryo morphology related variables: number of cells, fragmentation rate and embryo score on day 3 of embryo culture. CC were analysed for the expression of Hyaluronan synthase 2 (HAS2), Syndecan 4 (SDC4), Activated leukocyte cell adhesion molecule (ALCAM), Versican (VCAN), Prostaglandin-endoperoxide synthase 1 and 2 (PTGS1 and PTGS2), Grem-1 (GREM1), Dual specificity phosphatase 16 (DUSP16), Sprouty homolog 4 (SPROUTY4) and Ribosomal protein S6 kinase, 90 kDa, polypeptide 2 (RPS6KA2).

The expression profiles in cumulus cells from both GnRH analogue regimens were compared using a stepwise multivariate regression analysis allowing corrections for inter-patient variations using the patient and cycle characteristics.

Results: Inter-patient expression variances for the selected genes were comparable in both stimulation protocols. In long GnRH agonist cycles, the inter-patient variance was related to patient and cycle characteristics for 8/10 genes, while this was the case for only 3/10 genes in the short GnRH antagonist cycles. As a consequence a reliable multiple regression model (p < 0.002), explanatory for the gene expression level (e.g.: VCAN expression in agonist cycles = x + a*Age) - b (Ovarian Response) - c(Oocyte maturity), allowing the most exact comparison between the two treatments was only obtained for VCAN and SPROUTY4. VCAN expression lower in group B than group A but FF leptin levels were significantly lower in patients who subsequently became pregnant. Serum and FF leptin levels were negatively correlated with subsequent pregnancy (r = -0.333211; p = 0.0055 and r = -0.299381; p = 0.0112, respectively). Moreover, serum but not FF leptin positively correlated with BMI (r = 0.242; p = 0.048 and r = 0.043; p = 0.72). Initial FSH and E2 levels were equal in the two groups, while levels of E2 at the time of hCG application was significantly higher in patients destined to become pregnant.

The number of mature oocytes (MII) correlated negatively with age (r = -0.325; p = 0.018), and initial E2 levels (r = -0.355; p = 0.009) and positively with final E2 concentration (r = 0.72; p = 0.001). Fertility rate (number of 2 Pronuclei oocytes) correlate negatively with serum leptin levels (r = -0.438; p = 0.001) and follicular leptin levels (r = -0.471; p = 0.004) and positively with final E2 levels (r = 0.597; p = 0.0001).

Conclusions: Our results indicate that higher FF leptin levels are negatively correlated with IVF success rates. Strong correlation between serum and FF leptin suggests the former could be a source of follicular leptin. However, function of high FF leptin levels remains an outstanding question.

Clearly, leptin appears to be a marker of IVF success and a potential regulator of follicular steroidogenesis. Whether it exerts direct pathological effects on the developing oocyte, or simply is a marker for the myriad of harmful reproductive effects of obesity remains to be discerned.
was dependent on patients’ age, ovarian response and oocyte maturity. SPROUTY4 expression depended on age, serum FSH and fragmentation rate. Expression dynamics were different in both stimulation protocols. After a long GnRH agonist stimulation, COCs including an immature oocyte had higher VCAN expression in the CC and higher serum FSH levels related to higher SPROUTY4 expression in the CC. These relationships were less pronounced or absent in the antagonist cycles. The GnRH antagonist stimulation resulted in an overall lower SPROUTY4 expression in the CC than the long agonist cycles.

Conclusions: The short GnRH antagonist protocol generates an inter-patient variance in the COCs that can not be explained by the patient and cycle related characteristics considered in this analysis such as serum levels of endogenous LH or progesterone.

Correlations found between CC gene expression and patient and cycle characteristics for GnRH agonist cycles could not be confirmed in GnRH antagonist cycles. Other factors, not considered in our analysis, seem to influence the gene expression in cumulus cells from antagonist cycles.

For the considered gene set a simple extrapolation of the gene correlation (for instance oocyte competence) as found in agonist cycles cannot be done in antagonist cycles.

P-285 Influence of office hysteroscopy timing on pregnancy rates


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Objective: We hypothesized that the office hysteroscopy (OH) procedure performed during or close to an in vitro fertilization (IVF) cycle might have a favorable effect on pregnancy rates. The aim of the study was to evaluate the impact OH procedure timing on pregnancy rates of an IVF cycle.

Materials and Methods: In this retrospective study, 1258 women undergoing IVF treatment were assigned into three groups: group 1 (n = 407), group 2 (n = 280) and group 3 (n = 571) consisted of women to whom OH was performed within 50 days of IVF cycle, 51days-6 months and more than 6 months respectively. Implantation rates, pregnancy rates and clinical pregnancy rates were compared for each group.

Results: There was no difference with respect to age, duration of infertility, basal hormonal parameters, the treatment protocol and cycle parameters between the groups. However, the implantation rates were 22.1%, 16.1% and 11.1% in group 1, 2 and 3 respectively. Overall pregnancy rates were determined as 48.1%, 38.9% and 29.9% in group 1, 2 and 3 respectively. Clinical pregnancy rates were found to be 45.2%, 34.3% and 27.1% in group 1, 2 and 3 respectively. Implantation rates, pregnancy rates and clinical pregnancy rates were significantly higher in group 1 compared to group 2 and 3.

Conclusion: Performing OH during or close to an IVF cycle (within 50 days) has significant value in improving pregnancy rates. This enhanced implantation and pregnancy rates may be due to local stimulation effect of OH on endometrium.

P-286 NMR-based metabolomics reveals differently expressed metabolites in follicular fluid of pcos women: potential biomarkers for good quality oocyte?

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Introduction: Follicular fluid (FF) forms the microenvironment of the developing oocyte and has a direct bearing on oocyte quality, sperm–oocyte interaction, sperm-mediated oocyte activation, implantation and early embryo development. There is increasing interest in investigating whether the metabolic composition of the FF can provide information on the quality and developmental competence of female gametes. NMR-based metabolomics is becoming increasingly popular as NMR is capable of simultaneously detecting a wide variety of metabolites with accuracy and reproducibility. Further, it is non-destructive, cost-effective, rapid and requires minimal/no sample preparation. There is only one report on NMR-based metabolomic analysis of human FF where oocyte donors were included to identify the metabolites in the normal follicular environment. PCOS, a common cause of infertility, is often associated with anovulation due to endocrine and metabolic disorders. In the present study, NMR based metabolomic analysis has been carried out, for the first time, in FF of women with PCOS to identify differently expressed metabolites in the follicular microenvironment which may adversely affect IVF outcome.

Material and Methods: We recruited 10 PCOS (Group-1) and 10 tubal factor infertility women (Controls, Group-2) reporting for in-vitro fertilization and embryo transfer (IVF-ET) at the Institute of Reproductive Medicine, Kolkata, India. Institutional ethical clearance and informed patient consent were obtained before onset of the study. FF samples were centrifuged immediately at 1500 rpm for 10 min and cellular components removed.

Centrifuged 200 μl FF supernatant was mixed with 400 μl deuterium oxide (D2O) containing 1 mM International reference standard sodium salt of 3-(trimethylsilyl) propionic-2,2,3,3,d4 acid (TSP). The proton NMR spectra were recorded on Bruker 700MHz proton frequency spectrometer using CPMG pulse sequence and the spectra adjusted with reference to TSP peak set at zero. Further, phase and baseline correction were made prior to normalization of the data. Individual CPMG spectra (10.00 ppm to 0.40 ppm) were binned into 0.04 ppm. TSP and water were excluded in the binning process. Pareto-scaled and mean centered normalized binned data was applied to uni (t-test) and multivariate statistical methods [Principle Components Analysis (PCA) and Partial Least Squares discriminant analysis (PLS-DA)] to identify differently expressed FF metabolite biomarker(s) in PCOS women undergoing IVF. All statistics in this study were carried out using online metabolomics data analysis software, MetaboAnalyst.

Results: The univariate confirmatory statistical analysis t-test on binned data of FF was identified. 11 differently expressed metabolites which were identified using HMDB were 2-hydroxybutyric acid, Isocitric acid, Arginine, Leucine, Ornithine, Lysine, Proline, a-Glucose, b-Glucose, Taurine and Valine (P < 0.05). Furthermore, PCA score and loading plots showed different expression of metabolites in both the groups (PCI 72.3% and PC2 15.5%). PLS-DA score and loading plot indicated differently expressed metabolites in each group [(Component 1 (45.3%) and Component 2 (42.2%)]. IVF outcome parameters including fertilization rate, embryo formation and pregnancy outcome were observed to be adversely affected in women with PCOS as compared to controls. In PCOS, fertilization rate, embryo formation and pregnancy outcome was 62.09%, 58.44% and 23.7%, respectively. The controls group had improved outcome parameters; 81.25%, 76.92% and 37.5% for fertilization rate, embryo formation and pregnancy outcome, respectively.

Conclusion: 11 metabolites were differently expressed in FF of women with PCOS when compared with controls. These metabolites in the oocytes’ follicular microenvironment may be one of the key factors responsible for poor oocyte quality, which, in turn, affects viable embryo formation. This hypothesis is supported by the poor IVF outcome parameters observed in PCOS compared with controls. Some of these identified metabolites might be useful as biomarkers describing the state of follicular maturation, thereby permitting selection of the higher fertilization potential oocytes for increasing IVF pregnancy rates.

P-287 Predictive factors of success for intra-uterine insemination: a single centre retrospective study

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Introduction: Intra-Uterine Insemination (IUI) with or without stimulation is the most common assisted reproductive technique (ART) in the world. There is good evidence of the efficacy and cost-effectiveness of stimulated IUI (SO-IUI). However, parameters affecting its success have not been consistently reported. Here, we aimed to conduct a retrospective study in a cohort of patients
undergoing SO-IUI in KKIVF Centre (Singapore) on the factors influencing pregnancy rates (PR).

**Material and Methods:** We retrospectively analysed 851 SO-IUI cycles from 640 patients over a period of three years. These women underwent ovarian stimulation with either clomiphene citrate alone, a combination of clomiphene citrate and recombinant FSH (rFSH) or rFSH alone. An hCG injection was administered when at least 1 follicle was ≥16 mm, followed by IUI 36 h later.

**Results:** The overall PR was 16%. Factors associated with the highest PR were maternal age <35 y.o. (17.8% vs 11.8% for patient ≥35 y.o), where indication for IUI was sexual dysfunction, ovulation disorders or unexplained infertility (PR = 35.3%, 23.9% and 24.4% respectively), when more than 1 mature follicle was achieved (PR = 15.0% with 1, 19.0% with 2 and 20.3% with ≥3 follicles), where inseminated motile sperm was ≥1 million (16.7% vs 2.3% if less than 1 million) and when the sperm morphology was ≥% 1 (17% vs 5.4%)(Krujer Criteria).

**Conclusion:** Despite the tremendous increase in demand for IVF, SO-IUI still has an important role to play in ART with success rates of over 20% in certain groups of patients such as sexual dysfunction, ovulation disorders, unexplained infertility, mild male factor and when stimulation allows the maturation of more than 1 follicle.

**P-288 Have the parameters of concern during embryo transfer any prognostic relevance?**

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**Introduction:** Although a crucial moment in a cycle of IVF/ICSI, embryo transfer (ET) lacks standardization and important methodological differences persist. A difficult ET is usually defined by the presence of resistance to the entrance in the uterine cavity and/or the need to change embryos to a second catheter. In this study we analysed the implications of the occurrence of those problems in the treatment cycle outcome in our population.

**Material and Methods:** Retrospective evaluation of data between 2005 to 2010. Inclusion criteria were 1) IVF and ICSI cycles using long protocol with buserelin started in the initial follicular phase plus recombinant or urinary FSH and 2) at least one good embryo transferred. In only 2% of cases more than 2 embryos were transferred. ETs were performed with neither ultrasound guidance nor mock transfer, by a total of five clinicians. Previous information about cervical canal trajectory and hysteroscopy was taken into account in order to place the tip of the catheter at a 2 cm distance of the uterine fundus. Parameters studied: global classification as easy or difficult ET, need to use a forceps to pull the cervix, need to use a harder catheter with a guide as first approach, need to use a harder catheter, need to re-transfer retained embryo(s). The endpoint was clinical pregnancy rate (PR). Chi-square and Fisher’s exact test were used.

**Results:** 814 embryo transfers fulfilled the inclusion criteria. 721 were classified as easy and 93 as difficult. CPR was 48.4% and 38.7%, respectively (p = 0.1; NS). In 166 cases the use of a forceps pulling the cervix was registered (CPR of 39.2% vs 49.9% in those that did not require its use). The difference was statistically significant (p = 0.0145; RR = 0.706; CI 0.534-0.933). In 31 cases there was a need to change embryos to another catheter (CPR 35.5% vs 47.9% in the cases with no such need; p = 0.2; NS). In 15 occasions one or two retained embryo(s) were re-transferred (9 clinical pregnancies occurred in this small subgroup, which is not significantly different from 47.2% of CPR in those without retained embryos). In 199 ETs a hard catheter with a guide was used as the first choice; the CPR was 37.2% compared to 51.2% in those cycles with a soft catheter as the first option. This difference is significant (P = 0.0007; RR = 0.652; CI 0.508-0.837).

**Conclusions:** All efforts must be done to avoid the need to pull the cervix and an extra-detailed care is mandatory when the initial option must be a hard catheter since those circumstances correlated with a significant decrease in the clinical pregnancy rates.

**In our population the need to change embryos to another catheter or to re-transfer retained embryos did not seem to have impact on clinical pregnancy rates. However, numbers of such cases are small.**

**P-289 Spontaneous pregnancy in overweight plus obese women, with or without the polycystic ovary syndrome, after weight loss**

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**Introduction:** Over the last decades, obesity has become a worldwide spreading epidemic disease in developing countries. Obesity is a multifactorial, chronic inflammatory disease, with metabolic and endocrine disorders associated with derangements of behavior and socioeconomic consequences. It has been found that the main factors that influence the expression of obesity are environmental, up to 40%, and genetic, up to 60%. It has also been supported that besides the long-term risks of diabetes mellitus, cardiovascular disease, and cancer, obesity poses immediate threats for human reproduction. According to epidemiologic and experimental data, obesity is associated with subfertility and poor reproductive outcome in both, men and women. Especially in women, obesity is related to early and late pregnancy adverse outcomes. Furthermore, obesity seems to complicate another female disease entity, the polycystic ovary syndrome (PCOS). PCOS is the common cause of anovulatory infertility, affecting 5-10% of reproductive-aged women, and coexists with obesity in 38-88% of cases. It has been found that weight loss of 5-10% can restore ovulation in oligo- or anovulatory women and can further improve the total reproductive function. Thus, overweight and obese women who wish to have children are first advised to lose weight, and certainly if they are PCOS patients. This prospective study was designed with the aim to evaluate the pregnancy success rate after weight loss, via hypocaloric diet, physical exercise, and orlistat administration, but no other medical intervention, in overweight and obese women with or without PCOS.

**Material and Methods:** Twenty (20) overweight and obese women (aged 28.35 ± 0.88 years) with PCOS (group A), diagnosed based on the revised Rotterdam (2003) criteria and 19 overweight and obese women (aged 31.37 ± 0.85 years) without the syndrome (controls) (group B) were included in the study. Infertility duration ranged from one to seven years. There was no cervical or tube factor problems and male partner sperm was normal. All women were subjected to hypocaloric diet (basic metabolic rate -600 calories per day), physical exercise (one hour for three times per week) and orlistat administration (120mg, three times daily, before every meal), for a total period of six months. There was regular monthly follow-up for anthropometric evaluation and psychological support. The basal (start of treatment) hematological, endocrinological, biochemical (including oral glucose tolerance test) and ultrasonographic evaluation was repeated after the end of the third and the sixth month (end of treatment) of the study. The Free Androgen Index (FAI), the homeostasis model for insulin resistance (HoMA-IR), and glucose to insulin ratio (GIR) were also calculated.

**Results:** i) Ten out of the twenty women with PCOS (50%) and twelve out of the nineteen controls (63.2%) attained spontaneous pregnancy; ii) the total weight loss in women of group A was significantly higher that those of group B; iii) there were no significant differences in anthropometric, hormonal, biochemical and ultrasonographic parameters among women that succeeded pregnancy and those that did not succeed in every study group; iv) women with PCOS that attained pregnancy, besides the anthropometric, hormonal and biochemical changes, noted significant decrease in insulin resistance and recovery of normal menstrual function.

**Conclusions:** i) Even a moderate weight loss of 5-10% is sufficient to restore ovulation in many women without the polycystic ovary syndrome; ii) a higher weight loss is necessary for overweight and obese women with PCOS to attain pregnancy, due to the insulin resistance caused both by obesity and the syndrome, compared to those overweight and obese women without the syndrome; iii) insulin resistance in women with PCOS seems to be significant causative factor of the reproductive disorder affecting the function of hypothalamic-pituitary-ovarian axis.Eptation natibus ex et ute explobar am nonsereris cus
P-290 Anti-Mullerian Hormone (AMH) levels in serum and follicular fluid as predictors of ovarian response in stimulated (IVF and ICSI) cycles

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Introduction: Anti-Mullerian Hormone (AMH) was recently introduced as a marker of ovarian reserve in assisted reproduction. The cutoff values of AMH for prediction of poor response have not yet been determined.

Material and Methods: Ninety women were prospectively included in this clinical, non-interventional study. Baseline AMH, Follicle Stimulating Hormone (FSH) and Antral Follicle Count (AFC) were measured before starting ovarian stimulation. AMH was also measured on day 5 of stimulation and in the follicular fluid of the first aspirated follicle. The predictive value of baseline AMH, day 5 AMH and follicular fluid AMH was assessed comparatively to FSH and AFC for ovarian response. Ovarian response was defined as poor (< 4 oocytes), high (> 12 oocytes) or normal (≥ 4 oocytes and ≤ 12 oocytes). However, only 3 patients met the criterion for high ovarian response and thus analysis was focused on the prediction of poor response.

Results: Significant differences were present between poor responders and non poor responders regarding FSH (p = 0.019), baseline AMH (p = 0.002), AFC (p < 0.001), day 5 AMH (p = 0.005) but not for follicular AMH (p = 0.183). The largest AUC (Area Under the Curve) for poor ovarian response was obtained by AFC (AUC = 0.81) followed by baseline AMH (AUC = 0.70). At a level below 2.74 ng/mL the sensitivity of baseline AMH for prediction of poor response is 69% and specificity is 70.5%.

Conclusion: Baseline AMH is almost as good predictor for poor ovarian response as AFC.

P-291 Oocyte donors: why not avoid the ovarian hyperstimulation syndrome?

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Introduction: The aim of this study is to evaluate the effectiveness of the GnRH agonists in the induction of the ovulation to prevent the ovarian hyperstimulation syndrome (OHSS) in oocyte donors.

Material and Methods: We present a retrospective study of the cycles of oocyte donation performed in our clinic during 2010. We evaluated the incidence of OHSS depending on the protocol used to trigger the ovulation. The total dose of FSH needed, the level of Estradiol in serum the day of the trigger and the number of oocytes retrieved were also evaluated.

All our donors followed up a stimulation protocol starting in the second day of their menstrual cycles with a variable dose of FSH (Puregon, Organon, Spain) and 0.25 mg/day of Ganirelix (Orgalutran, Organon, Spain) according to the multiple-dose, flexible protocol. To induce the final oocyte maturation, 0.2mg of subcutaneous Triptorelin or 250 mg of HCG was supplied.

OHSS was defined as abdominal discomfort or pain, and presence of ascitis after the ovulation retrieval.

Results: Of the 161 cycles of oocyte donation carried out in our clinic in 2010, in 52 cases (32.3%) the ovulation was triggered using HCG and in 109 (67.7%) using triptorelin.

No significant differences were found in the age of the donors (24.15 ± 3.53 years in the group of HCG; 24.06 ± 3.15 years in the group of triptorelin), in the total dose of FSH required (1752.40 ± 483.12 UI Vs. 1799.43 ± 603.89UI), nor in the Estradiol level the day of the triggering (2390.60 pg/mL Vs. 2329.58 pg/mL) neither in the number of oocytes retrieved (13.83 ± 6.66 Vs. 16.30 ± 8.07).

The incidence of OHSS was 19.2% in the group of HCG and 1.8% in the group of GnRH agonist.

Conclusion: Using GnRH agonists for the final oocyte maturation, when using an ovarian stimulation protocol with GnRH antagonists, is an effective strategy to avoid OHSS in oocyte donors. According to this, nowadays we must use the tools we have to reduce the OHSS risk to the minimum.

P-292 Mayer-Rokitansky-Kuster-Hauser Syndrome (MRKHS) & Surrogate IVF performance in typical and atypical forms

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Introduction: The Mayer–Rokitansky–Kuster–Hauser syndrome (MRKH) is a congenital malformation of the female genitals as a result of interrupted embryonic development of the Müllerian (paramesonephric) ducts. Associated malformations frequently accompany the genital malformations being recently classified as the typical form (isolated urovaginal aplasia/hypoplasia) and the atypical form (additional malformations in the ovary or renal system). Surrogacy is an efficient option for procreation in patients with MRKHS. The aim of the study was to compare the IVF performance of patients with typical and atypical forms of MRKHS, in order to find which type is more favorable for conception.

Material and Methods: Between January 2000 and December 2010, a total of 102 cycles of surrogate IVF were initiated in 27 treated MRKHS patients: 20 patients with typical form performed 72 IVF cycles compared with 7 patients with atypical form performing 30 IVF cycles. The gametes of the infertile couple were used and the embryos were transferred to the surrogate uterus. The MRKHS patients were stimulated with a long protocol started at the mid-luteal phase. The surrogates’ endometrium was prepared, in parallel, by estradiol valerate and vaginal micronized progesterone. The various examined parameters of the biological mother were: age, hormonal profile during COH and laboratory outcome. Statistical analysis was performed by student’s t-test and chi-square, as appropriate.

Results: The mean age of the typical MRKHS patients was 32 ± 4.7y, and of atypical patients 30 ± 3.1y. The mean number of gonadotrophins needed for stimulation and treatment duration was significantly higher in the atypical form (3600 ± 1294 IU for 13 ± 2.3days compared with 2925 ± 967 IU for 11.6 ± 1.6 days, p ≤ 0.01). Serum E2 and P level on HCG administration day were similar between the above two types. A significantly higher mean number of follicles 12.6 ± 6; 8.9 ± 5.4 p ≤ 0.03, MII oocytes 8.7 ± 5.1; 6.7 ± 4.8 p ≤ 0.05, fertilizations 6 ± 3.6; 4.4 ± 3.3 p ≤ 0.03 and cleaving embryos 5.7 ± 3.8; 4.1 ± 3.3 p ≤ 0.01 were available in the typical patients compared with the atypical patients, respectively. No significant difference was observed concerning fertilization rate, cleavage rate and the mean number of transferred embryos. embryo quality of the transferred embryos and pregnancy rate per cycle were also similar between the two groups.

Conclusions: The typical form of MRKH is more favorable for conception since less gonadotrophins are needed for shorter duration are needed for ovarian hyperstimulation. The mean number of follicles, oocytes, MII oocytes, fertilizations and cleaving embryos are also higher in the typical form. Pregnancy rates are similar since the number and quality of transferred embryos was comparable.

P-293 Decreased expression of endometrial L-selectin ligand during the midluteal phase in female smokers

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Introduction: The L-selectin-ligand system may play a role in the embryo implantation process. The maternal smoking habit downregulates, in a dose-dependent manner, cytotrophoblast L-selectin ligand (LSL) expression. LSL is expressed in the endometrium during early-midsecretory phases of menstrual cycle. The objective of this study was to assess the effect of smoking habit on the endometrial L-selectin ligand during the uterine receptivity period.

Material and Method: Four infertile women smoking more than ten cigarettes daily were double paired by age and body max index (BMI) and compared with non-smoker infertile women presenting the same reproductive pathology and with fertile non-smoker women. An endometrial sample was obtained by curette on day 7 after ovulation timed by urinary LH and transvaginal ultrasound. Immunohistochemistry was used to determine the localization and L-selectin ligand expression measured semiquantitatively by HSCORE.
P-294 The ADHL3A2 as marker of lipid oxidative stress in granulosa-lutein cells and its relationship with IVF outcome

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Introduction: Fatty aldehyde dehydrogenase (ADHL3A2) is involved in the detoxification of aldehydes derived from lipid peroxidation and in response to endoplastic reticulum stress (ERS). The ERS response plays a crucial role in the pathogenesis of the metabolic syndrome, diabetes mellitus, obesity and neurodegenerative disorders. We have shown that the ADHL3A2 gene expression in granulosa-lutein cells is upregulated in women above 40 years old as compared to egg donors with a mean age of 22 years old. The present study was designed to study ADHL3A2 expression in patients with different origin of infertility and its correlation with the IVF outcome.

Material and Methods: 100 women undergoing IVF were classified as: 1. Patient above 40 y/o with good ovarian reserve and tubal or male factor infertility (“No ovarian factor” > 40 y/o; NOF = 24); 3. Endometriosis (ENDO; n = 19); 4. Poor responders (PR; n = 18); 5. Polycystic ovary syndrome (PCOS; n = 18). Ovulation induction was carried out using long or microflare or antagonist protocol based on clinical parameters and gonadotrophin doses were selected based on ovarian reserve (day 3 FSH and E2 and basal antral follicle count) and adjusted to the individual response. After ultrasound guided egg retrieval, mural GL cells were isolated from pooled follicular fluids from each patient using a percoll gradient and anti-CD45 immunobeads to eliminate WBCs, viability was assessed by trypan blue. ADHL3A2 was measured by RT-PCR as relative expression compared to beta actin. Statistical analysis was performed with the SPSS using Students t-test.

Results: ADHL3A2 gene expression is significantly higher in patients with NOF > 40 y/o (5906 ± 804) and in PR (4381 ± 537) compared to ED (2659 ± 399), EM (2982 ± 428) and PCOS (2182 ± 312) (p < 0.05). Considering the totality of patients studied a statistically significant difference in ADHL3A2 gene expression was found between pregnant and not pregnant patients (2938 ± 309 vs 4318 ± 428, p = 0.016).

Conclusions: Patients above 40 y/o and PR present higher ADHL3A2 expression, suggesting an increased level of lipotoxicity and ERS in these women. The different ADHL3A2 expression in pregnant vs not pregnant women establishes a direct relationship between lipidic oxidative stress and the IVF outcome.

P-295 Obstetric outcomes in oocyte donor pregnancy

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Introduction: The history of oocyte donation is relatively young in the framework of In Vitro Fertilization (IVF) techniques and little has been discussed about the obstetric outcomes of oocyte donor pregnancy. The aim of the study is to assess obstetric outcomes of pregnancy following in vitro fertilization with embryo transfer (IVFET) using donor oocytes, and to compare them with those from autologous IVFET and with spontaneous pregnancy from women with advanced age (AMA), to identify possible criticisms and help in counselling women and their doctors.

Material and Methods: The study included a total of 70 pregnancies delivered, at different gestational age, in our Institute, between December 2008 and September 2010. Fourteen from oocyte donor FIVET (d-FIVET), aged 32-52 years, were the study group. Those results were compared with the two next consecutive deliveries from autologous FIVET (FIVET group), (n = 28; age 30-46 years), and with two next consecutive deliveries from women older than 40 years (Advanced Maternal Age: AMA) (n = 28, age 40-45 years). We evaluated the occurrence of Pregnancy-induced Hypertension (PIH), Preeclampsia (PE), Fetal Growth Restriction (IUGR), Gestational Diabetes (GDM), Preterm premature rupture of membranes (pPROM), Preterm Birth, Gestational age at birth, Placental anomalies, Mode of delivery, Birthweight and neonatal Appgar score. Fetal weight are corrected with gestational age at delivery according to Gardosi. Statistical analysis was performed with the chi-square test.

Results: Oocyte donor pregnancies had significantly higher rates of PE (d-FIVET 21.4%, FIVET 0%, AMA 0%, p < 0.011). They also had higher rates of PIH than FIVET (d-FIVET 21.4%, FIVET 0%, p < 0.011). We found also placental anomalies only in the d-FIVET group: the incidence of placental accretion is 28.6%, p < 0.003. Hypertensive disorders were surprisingly not related to maternal age or other in vitro fertilization technique.

Conclusions: This study is the first that compares obstetric outcomes of donor pregnancy to autologous FIVET and to advanced maternal age, this last given that most women requiring oocyte donation are elders.

Obstetricians who deal with pregnancies from oocyte donation should be aware of the worse obstetric outcomes, especially placenta accrete and pregnancy-related hypertensive disorders, and warrant a close blood pressure monitoring and accurate placenta ultrasound.

Pregnancy is an immunological paradox: the semi-allogenic fetus has an immunologic privilege. The mother allows its implantation and growth. In oocyte donor pregnancy something unique happens: both paternal and maternal semen are foreign for mother. A “wrong” interaction among mother and fetus could explain the abnormal placentation, also as a cause of hypertensive disorders. All women who conceive pregnancy through oocyte donation should be counseled since the pre-conception period and referred to specific Centers for high risk pregnancy.

P-296 Human ovarian ageing: a role of advanced glycated endproducts (AGE) and the receptor RAGE?

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Introduction: Reproductive senescence is characterized by depletion of ovarian follicles and emergence of meiotic errors in oocytes. The molecular mechanisms compromising ageing granulosa cells and oocytes are poorly understood. It is uncertain how ovarian dysfunction is related to the general processes of cellular ageing, such as accumulation of unrepaird DNA damage, telomere loss, deterioration of mitochondrial function, and accumulation of altered proteins.

Advanced glycated endproducts (AGE) are adducts formed by non-enzymatic glycation and oxidation of proteins and lipids in long-lived tissues. Their formation is accelerated by ageing and oxidative reactions, and accumulation of AGE-modified proteins in the brain, blood vessels, and connective tissue is shown to contribute to the pathogenesis of atherosclerosis, Alzheimer’s disease, and diabetes. Advanced glycation may cause tissue injury by direct modification of cellular proteins. Furthermore, indirect AGE-induced damage may arise by binding of AGE-modified soluble proteins to specific cellular...
receptors, including the receptor of advanced glycation end products (RAGE). RAGE belongs to the immunoglobulin superfamily of transmembrane receptors, and is expressed by various cell types, including endothelial and immune cells. Ligand binding by RAGE has been shown to activate monocytes, increase expression of cell adhesion molecules and cytokines, and so promote an inflammatory reaction that contributes to tissue injury.

Since AGE-modified proteins are present in the preovulatory follicle, we examined whether AGE and its receptor RAGE could be involved in cellular dysfunction during ovarian ageing.

**Methods and Results:** Follicular fluid was obtained from women undergoing assisted reproduction treatment. Mononuclear cells were isolated using gradient centrifugation and were labeled with fluorochrome-conjugated antibodies for CD45 and CD14 in order to distinguish granulosa lutein (GL) cells from leukocytes. Cells were further labeled with anti-AGE antibody and examined by multicolor immunofluorescence and flow cytometry. By analyzing permeabilized and non-permeabilized cells, the presence of AGE was affirmed on the surface and in the cytoplasm of GL cells. Intrafollicular monocytes, but not lymphocytes, were also AGE positive. Using gel electrophoresis and Western blot of GL cell extracts, we observed various AGE-modified protein species.

In order to examine whether ovarian cells express RAGE that would allow specific AGE binding on the cell surface, cells were labeled with anti-AGE antibody. Both GL cells and intrafollicular monocytes, but not lymphocytes, were found to express RAGE. Specific RAGE-related fluorescence intensity correlated with the patients’ chronological age (GL cells, n = 7, r = 0.93, P < 0.005; monocytes, n = 7, r = 0.54, P = 0.27).

Cells expressing RAGE may bind **AGE in vitro.** To examine this possibility, we conjugated AGE-albumin and albumin with fluorescein, and incubated follicular fluid-derived cells with these probes **in vitro.** GL cells and intrafollicular monocytes, but not lymphocytes, were found to preferentially bind AGE-albumin. Furthermore, binding of AGE-albumin by GL cells tended to correlate with patients’ chronological age (n = 9, r = 0.39, P = 0.30).

**Conclusions:** The presence of AGE-modified proteins on the surface of freshly isolated GL cells and intrafollicular monocytes suggests that ovarian cells are exposed to AGE-related damage **in vivo.** A possible cellular mechanism is activation of AGE receptor RAGE, which appears to be expressed by GL cells and ovarian monocytes in an age-dependent manner. Supporting such a role of RAGE, GL cells and monocytes were found to bind AGE-albumin **in vitro,** indicating the presence of unoccupied AGE binding sites on the surface of ovarian cells. Although the downstream effects of RAGE activation need to be explored, we propose that AGE-modified proteins and the receptor RAGE promote an ageing-related dysfunction of ovarian cells.

**P-298** GnRH antagonist protocol followed by frozen-thawed blastocyst transfer with long zona dissection can maximize cumulative pregnancy rates per retrieval without OHSS

**Introduction:** We devised a new strategy, GnRH antagonist protocol with GnRH agonist trigger followed by frozen-thawed blastocyst transfer with long zona dissection. The purpose is to investigate the effects of this new strategy according to age.

**Material and Methods:** Inclusion criteria of patients were [1] basal follicle-stimulating hormone (FSH) level < 10 mIU/mL, [2] normal uterine cavity and [4] adequate sperm for IVF or ICSI. Thirty-two women aged < 35 (group A) and 14 women aged 35-39 (group B) were undergone GnRH antagonist protocol and GnRH agonist trigger for the prevention of early-onset OHSS and the obtaining of many oocytes. All oocytes cultured to blastocyst stage and all blastocysts grade 3BB were cryopreserved. Fresh embryo transfers were not performed for the complete prevention of late-onset OHSS and the overcome of embryos-endometrium dysynchrony. Long zona dissection just before frozen-thawed blastocyst transfers were performed for complete hatching and high implantation.

**Result(s):** The average numbers of retrieved oocytes and blastocysts grade 3BB were 13.5 ± 5.1 and 4.8 ± 2.6 in group A and 11.7 ± 7.8 and 2.9 ± 2.2 in group B, respectively, and OHSS was not occur in all women. Implantation rates were 43.2% in group A and 35.7% in group B. Clinical pregnancy rates per transfer were 68.4% in group A and 52.9% in group B. Cumulative pregnancy rates per retrieval were 81.3% in group A and 64.3% in group B.

**Conclusion(s):** GnRH antagonist protocol with GnRH agonist trigger followed by frozen-thawed blastocyst transfer with long zona dissection can prevent OHSS and maximize cumulative pregnancy rates per retrieval. Especially, the number of blastocysts grade 3BB, implantation and pregnancy rates were significantly higher in women aged < 35 compared with women aged 35-39.

**P-299** Twice-daily assessments of the local tolerability associated with a new MENOPUR multi-dose formulation during controlled ovarian stimulation

**Introduction:** This prospective investigation of local tolerability was included in the MEGASET (MENOPUR in GnRH Antagonist Cycles with Single Embryo Transfer) study, a randomised, controlled trial in women undergoing controlled ovarian stimulation comparing the efficacy and safety of highly purified menotropin (MENOPUR, Ferring Pharmaceuticals) (N = 374) and recombinant FSH ( follitropin beta, PUREGON, MSD/Scheringer-Plough) (N = 375). MENOPUR was provided as a vial (powder) and pre-filled syringes (solvent for solution for injection) which after reconstitution delivered 1200 IU of FSH activity and 1200 IU of LH activity at 600 IU/mL concentration. PUREGON was provided as a cartridge containing 900 IU FSH in a 1.08 mL
We perform a prospective study on 28 consecutive patients undergoing IVF/ICSI treatment at the Reproductive Medicine Unit of the Clínica Alemana de Santiago, Chile. A classical ovarian stimulation protocol with follicle stimulating hormone (FSH) used either GnRH agonist or antagonist was carried out. On the day of embryo transfer (day 2 or 3 after ovum pick-up), we performed a transvaginal ultrasound scan using a Voluson E8 (GE Healthcare, Zipf, Austria). Women with uterine abnormalities were excluded. All measurements were performed by a single observer (PS). A longitudinal view of the uterus and endometrial cavity was obtained. The ultrasound machine was switched to the 3D mode with power Doppler using a scanning angle of 180° to ensure that a complete uterine volume was obtained. Volumes were stored and assessed in a personal computer by the same investigator (PS) who was blind to the final result regarding pregnancy. Three-dimensional endometrial volumetric and vascular measurements were determined using VOCAL program within 4D View. All measurements were performed manually in plane C (coronal image). Through the rotational technique with 9° rotation steps, 20 endometrial slices were obtained defining the endometrium. The power Doppler signal within it was quantified using the histogram facility, and the vascularization indexes (vascularization index; VI; flow index, FI; and vascularization flow index, VFI) were obtained. To assess the subendometrial region, we used shell-imaging to define a three-dimensional region within 5 mm of the originally defined endometrial contour and the power Doppler signal within this subendometrial region was evaluated, obtaining the vascularization indexes of this region. The primary outcome measure was clinical pregnancy. To statistics analysis, Kolmogorov-Smirnov’s test was used to establish if the samples were normally distributed. The paired t-test in the normally distributed samples, and Wilcoxon test in the case of non-normally distributed samples were used. The receiver operating characteristic (ROC) curve was applied to establish the predictive value of the parameters. The two-tailed value of P < 0.05 was considered statistically significant.

Results: There were no differences regarding patient’s age, baseline level of FSH, total amount of FSH doses, number of MII oocytes obtained, and number of embryos grade 1 or 2 transferred between patients who get pregnant (11/28) and who did not (17/28) (P > 0.05). Regarding endometrial flow indexes, there were no differences for VI (1.62 ± 2.7 v/s 1.61 ± 2.3), FI (22.82 ± 9.8 v/s 25.47 ± 5.8), and VFI (0.58 ± 0.1 v/s 0.52 ± 0.9) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was no statistically significant for VI (0.497), FI (0.552), and VFI (0.493). Regarding subendometrial flow indexes, there were no differences for VI (2.65 ± 4.1 v/s 3.68 ± 4.3), FI (25.78 ± 4.8 v/s 26.25 ± 3.8), and VFI (0.78 ± 1.3 v/s 1.08 ± 1.3) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was not statistically significant for VI (0.594), FI (0.506), and VFI (0.587).

Conclusions: The detection of endometrial and subendometrial blood flow by 3D power Doppler in IVF/ICSI cycles is not predictive of pregnancy outcome on IVF/ICSI patients when they are performed on day of embryo transfer.

P.300 3D power Doppler vascularization indexes are not predictive for pregnancy outcome on IVF/ICSI patients

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Introduction: Embryo implantation depends on embryo quality and endometrium receptivity. Ultrasonographic assessment of the endometrium allows a non-invasive method to evaluate endometrial receptivity on IVF/ICSI patients. Endometrial and subendometrial blood flow evaluated through power Doppler on the day of embryo transfer may predict pregnancy on these patients, however there is no enough evidence to support this. The aim of this study is to evaluate the role of the endometrial and subendometrial flow indexes measured through 3D power Doppler on the day of embryo transfer. Our hypothesis is that a higher blood flow can predict pregnancy on IVF/ICSI patients.

Material and Methods: We perform a prospective study on 28 consecutive volunteers patients undergoing IVF/ICSI treatment at the Reproductive Medicine Unit of the Clínica Alemana de Santiago, Chile. A classical ovarian hyperstimulation protocol with follicle stimulating hormone (FSH) using either GnRH agonist or antagonist was carried out. On the day of embryo transfer (day 2 or 3 after ovum pick-up), we performed a transvaginal ultrasound scan using a Voluson E8 (GE Healthcare, Zipf, Austria). Women with uterine abnormalities were excluded. All measurements were performed by a single observer (PS). A longitudinal view of the uterus and endometrial cavity was obtained. The ultrasound machine was switched to the 3D mode with power Doppler using a scanning angle of 180° to ensure that a complete uterine volume was obtained. Volumes were stored and assessed in a personal computer by the same investigator (PS) who was blind to the final result regarding pregnancy. Three-dimensional endometrial volumetric and vascular measurements were determined using VOCAL program within 4D View. All measurements were performed manually in plane C (coronal image). Through the rotational technique with 9° rotation steps, 20 endometrial slices were obtained defining the endometrium. The power Doppler signal within it was quantified using the histogram facility, and the vascularization indexes (vascularization index; VI; flow index, FI; and vascularization flow index, VFI) were obtained. To assess the subendometrial region, we used shell-imaging to define a three-dimensional region within 5 mm of the originally defined endometrial contour and the power Doppler signal within this subendometrial region was evaluated, obtaining the vascularization indexes of this region. The primary outcome measure was clinical pregnancy. To statistics analysis, Kolmogorov-Smirnov’s test was used to establish if the samples were normally distributed. The paired t-test in the normally distributed samples, and Wilcoxon test in the case of non-normally distributed samples were used. The receiver operating characteristic (ROC) curve was applied to establish the predictive value of the parameters. The two-tailed value of P < 0.05 was considered statistically significant.

Results: There were no differences regarding patient’s age, baseline level of FSH, total amount of FSH doses, number of MII oocytes obtained, and number of embryos grade 1 or 2 transferred between patients who get pregnant (11/28) and who did not (17/28) (P > 0.05). Regarding endometrial flow indexes, there were no differences for VI (1.62 ± 2.7 v/s 1.61 ± 2.3), FI (22.82 ± 9.8 v/s 25.47 ± 5.8), and VFI (0.58 ± 0.1 v/s 0.52 ± 0.9) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was no statistically significant for VI (0.497), FI (0.552), and VFI (0.493). Regarding subendometrial flow indexes, there were no differences for VI (2.65 ± 4.1 v/s 3.68 ± 4.3), FI (25.78 ± 4.8 v/s 26.25 ± 3.8), and VFI (0.78 ± 1.3 v/s 1.08 ± 1.3) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was not statistically significant for VI (0.594), FI (0.506), and VFI (0.587).

Conclusions: The detection of endometrial and subendometrial blood flow by 3D power Doppler in IVF/ICSI cycles is not predictive of pregnancy outcome on IVF/ICSI patients when they are performed on day of embryo transfer.

P.301 Smoking is associated with a reduced age of final pregnancy, consistent with a detrimental effect on ovarian reserve

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Introduction: Tobacco smoking has been implicated in causing an acceleration of ovarian ageing by reducing follicular reserve. This manifests in an earlier age at menopause. We hypothesised that reduced follicular reserve should also therefore impact on the age-profile of pregnancies experienced by smokers, perhaps reducing the age at final pregnancy. In this study we demonstrate that after correcting for clinical and socioeconomic confounders, the age of first and final pregnancy is lower in smokers compared to non-smokers.

Material and Methods: A University Medical Centre perinatal database of 50082 pregnancies in which clinical, lifestyle and socioeconomic features in the periconceptional period and perinatal outcomes were reported between 2002 and 2010 was interrogated. The population studied is known to be geographically stable and therefore the age of first and last pregnancy reported in the database are likely to represent the complete obstetric history of the women studied. The age at booking for first pregnancy and last pregnancy was analysed by t-test in the normal distribution. No other significance differences were found.

Results: There were no differences regarding patient’s age, baseline level of FSH, total amount of FSH doses, number of MII oocytes obtained, and number of embryos grade 1 or 2 transferred between patients who get pregnant (11/28) and who did not (17/28) (P > 0.05). Regarding endometrial flow indexes, there were no differences for VI (1.62 ± 2.7 v/s 1.61 ± 2.3), FI (22.82 ± 9.8 v/s 25.47 ± 5.8), and VFI (0.58 ± 0.1 v/s 0.52 ± 0.9) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was no statistically significant for VI (0.497), FI (0.552), and VFI (0.493). Regarding subendometrial flow indexes, there were no differences for VI (2.65 ± 4.1 v/s 3.68 ± 4.3), FI (25.78 ± 4.8 v/s 26.25 ± 3.8), and VFI (0.78 ± 1.3 v/s 1.08 ± 1.3) in non-pregnant group compared with pregnant group, respectively. The area under ROC curve was not statistically significant for VI (0.594), FI (0.506), and VFI (0.587).

Conclusions: The detection of endometrial and subendometrial blood flow by 3D power Doppler in IVF/ICSI cycles is not predictive of pregnancy outcome on IVF/ICSI patients when they are performed on day of embryo transfer.
Results: Data relating smoking behaviour to age of conceiving the first ongoing pregnancy was available from 17386 women, and to age of final reported ongoing pregnancy from 8042 women. The mean age (years ± SD) of booking for the first reported pregnancy in non-smokers was 27.7 ± 5.5, and 22.2 ± 5.3 in women smoking > 10 a day at the time of booking. The mean age of last reported pregnancy in each group, was 30.7 ± 5.2; and 26.6 ± 5.7 respectively. After controlling for confounders including socioeconomic class, the age of conceiving first and last pregnancy was observed to be significantly younger in active smokers than women who had never smoked: p < 0.05.

Conclusions: Smokers are shown to begin and end their reproductive life at an earlier age than non-smokers. While these data have been corrected for socioeconomic class we cannot exclude a possible impact of other unidentified confounding variables. While the factors which determine when a couple will start a family or stop extending it are complex, these data are consistent with an accelerated ovarian aging effect in smokers resulting in them conceiving their final pregnancy at a younger age.

P-302 Natural cycle IVF/M treatment for women with advanced maternal age and low ovarian reserve

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Introduction: Natural cycle IVF and In vitro maturation of oocytes (IVM) can be effective in avoiding ovarian hyperstimulation syndrome (OHSS). Mild approaches in ART with no or low stimulation regimens are increasingly used to make ART safer and affordable. However, IVM has been mainly used in women with PCO and PCOS. Recently, it has been reported that high pregnancy and live birth rates can be obtained when natural cycle IVF combined with in vitro maturation (IVM) of immature oocytes (natural cycle IVF/M) for younger women (under 35 years of age) with normal ovarian reserve (Lim et al. 2009). At our centre, we have conducted a pilot study with Natural cycle IVF with IVM in older women and in those with low ovarian reserve. Our initial results suggest that natural IIIVF/M can increase the number of embryos available for transfer with a potential to improve pregnancy rates in older women with low ovarian reserve.

Method: Ovulatory women with low ovarian reserve and older women with previous poor response to conventional stimulation were selected for natural cycle IVF/M. Baseline ultrasound scan was performed on day 3 of cycle, and on day 7 or 8 in order to determine the timing of human chorionic gonadotrophin (hCG) injection. When the leading follicle reached 13-14 mm in diameter with a minimum of 3 antral follicles (combined two ovaries) and endometrial thickness of 6 mm with triple layer morphology, 10,000 IU hCG was administered. Oocyte collection was performed under transvaginal ultrasound guidance for both leading and small follicles. The collection, identification, in vitro maturation, insemination, culture and transfer of embryos was performed as described by Lim et al.

Patients whose endometrium was less than 6 mm were given 6 mg of oestriadiol per day from the day of hCG injection, and 400 mg progesterone from the day of oocyte collection. A blood test (βhCG) was performed 14 days post oocyte collection, and clinical pregnancy was confirmed with ultrasound scan 2 weeks later.

Results: Fifteen Natural IVF/IVM cycles were undertaken on twelve patients with low ovarian reserve (mean age 39.4, mean total antral follicle count 3.5, mean AMH 3.1 and mean number previous failed stimulation cycles 4.2). A total of 14 oocytes were collected from 15 leading follicles of which 13 were mature (MI); 12 of these fertilised, with 100% cleavage rate. A total of 30 immature oocytes (mean number 2.3 per patient) were collected. 21 matured in –vitro (70%), 15 fertilised (71.4%) and 13 cleaved (86.6%). All patients had an embryo transfer (20 embryos – mean 1.33), resulting in an implantation rate of 15%, a pregnancy rate of 20% and an ongoing clinical pregnancy rate of 13.3%.

Conclusions: Although the patient group has low ovarian reserve and high mean maternal age, a good maturation, fertilisation and cleavage rates for IVM oocytes were achieved resulting in an increase in the number of embryos for transfer. We found that mature (MI) oocytes were obtained from 13-14 mm leading follicles with no spontaneous oulations. We conclude that natural cycle IVF/M has the potential to improve pregnancy rates in patients due to reduced ovarian reserve. Prospective randomised studies are required.

P-303 Neurotrophin 3 and its receptor tropomyosin-related kinase receptor C in human preantral follicles from fetuses, girls and women

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Introduction: Neuronal growth factors appear to play important roles in the early stages of folliculogenesis in mammalian ovaries including those from humans. The signals involved in growth initiation of primordial follicles are as yet unidentified, and neurotrophins (NTs) including NT3 might be involved in this process. Data regarding ovarian expression of NT3 and its tropomyosin-related kinase C (TrkC) receptor, is still sparse, particularly in human preantral follicles. The aim of this study was, therefore, to investigate the expression of the proteins for NT3 and TrkC and their mRNA transcripts in human ovarian samples from fetuses, girls and women.

Material and Methods: Fifteen ovarian samples from human fetuses aged 21-33 gestational weeks and 36 ovarian samples from girls and women aged 5-39 years were utilized for the present study. The specimens were fixed and prepared for paraffin sections. Sections were processed for immunohistochemical (IMH-protein detection) studies for detection of NT3 and TrkC and for in situ hybridization (ISH-mRNA transcript detection) studies for detection of the catalytic long isoform of TrkC. The IMH primary antibodies against NT3 and TrkC (all three isoforms) had no cross-reactivity with other NTs or Trk receptors, respectively. Custom-designed antisense phosphodiester DNA oligonucleotide probes were used for ISH. Reverse transcription polymerase chain reaction (RT-PCR) was conducted on total RNA extracted from frozen ovarian samples to identify transcripts of the two NT3 isoforms and three TrkC isoforms. Positive and negative controls were applied for the IMH, ISH and RT-PCR procedures. Our institutional ethics committee approved the study protocol, and every woman or minor’s parents signed an informed consent form.

Results: Positive staining for the NT3 protein was identified in oocytes and granulosa cells (GCs) of all samples tested (from primordial follicular stages onwards). NT3 protein staining was mostly weak in the fetal samples. TrkC protein staining was present in oocytes in 70% of the fetal samples and in GCs in 60% of the fetal samples, and in oocytes and GCs of all samples from girls/ women (from primordial follicular stages onwards). The mRNA transcripts for the full-length TrkC isoform were identified by ISH in oocytes in 60% of the fetal samples (weak intensity) and in all samples from girls/women and were present in GCs only in the samples from girls/women (from primordial follicular stages onwards). A portion of the stroma cells in all the samples expressed the proteins and mRNA transcripts for NT3 and TrkC. IMH (NT3: mouse skin sample) positive control and ISH positive controls (polyoxyethyleneimidate, β-actin and α-tubulin) stained positively. ISH negative controls (NT3: antibody absorption with its blocking peptide; TrkC: normal IgG antibodies) and ISH negative controls (random oligonucleotide sequences) did not stain positively. The two NT3 isoforms and the three TrkC isoforms (the catalytic long isoform and two truncated isoforms) were identified by RT-PCR in all samples tested. The positive RT-PCR control gene (α-actin) was positive in all the samples tested. RT-PCR negative controls (processed without RT) did not yield an amplification product.

Conclusions: The present study contributes novel information to the increasing evidence of the importance of neuronal growth factors in the mammalian ovary, specifically at early follicular stages. In rodents, NT3 has been identified as an inductor of primordial follicular activation. Therefore, our detection of NT3’s TrkC receptor in the human ovary, specifically in GCs of primordial follicles, suggests that NT3 might be involved in the primordial-to-primary transition also in humans. To elucidate the actual role of NT3 in the activation of human primordial follicles, it should be added in future studies to the culture medium.

P-304 CD11c+ HLADR+ dendritic cells are present in human ovarian follicular fluid and their maturity correlates with ovarian response to gonadotropins

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Conclusions: While the factors which determine when a couple will start a family or stop extending it are complex, these data are consistent with an accelerated ovarian aging effect in smokers resulting in them conceiving their final pregnancy at a younger age.
Introduction: Dendritic cells (DCs) are bone marrow derived innate immune cells specialized in the initiation and modulation of the adaptive immune response. Recently, DCs in the endometrium have been shown to enhance a local inflammatory reaction that correlates with implantation rates and pregnancy outcomes. The ovulatory process is initiated by an inflammatory phase involving leukocyte recruitment, leading to rupture of the follicle and terminated by a repair phase. We therefore sought to determine whether DCs are present in the ovarian follicular fluid (FF), and if their abundance or maturation state correlates with reproductive outcome.

Material and Methods: After obtaining informed consent according to the Institutional Review Board of our institution, FF samples were collected from 31 patients undergoing in vitro fertilization (IVF) cycles.

The primary diagnosis of infertility: male factor (12 patients), unexplained infertility (11 patients) unilateral/bilateral tubal occlusion (6 patients), and polycystic ovarian syndrome (PCOS) (2 patient).

The IVF treatment protocol comprised of standard ovarian hyperstimulation by a long luteal GnRH agonist, flare up GnRH agonist or GnRH antagonist protocols combined with recombiant FSH or purified urinary gonadotropins. Transvaginal oocyte retrieval was performed 34 hours after human chorionic gonadotropin (hCG) injection. The fluid of the first largest follicle in each ovary was aspirated and collected into separate tubes. FFs with overt bloody contamination were discarded and were not further analyzed.

FF cells were immunostained with fluorescent antibodies and analyzed by flow cytometry. DCs were identified as CD45+ CD11c+ HLA-DR+ cells. In order to assess DC maturity, mean fluorescent intensities (MFIs) and geometric means of individual histograms of HLA-DR expression on CD45+ CD11c+ cells were derived using CellQuest software.

Results: Other than granulosa cells, the cellular component of FF derived from IVF patients (n = 31) contained 10% CD45+ hematopoietic cells. Of this latter population, CD11c+ HLA-DR+ DCs comprised a significant fraction (15.6 ± 2.8%). Interestingly, the ovarian response to gonadotropins, as reflect by estradiol levels at the day of hCG administration, correlated positively with the maturity of FF DCs as reflected by the MFIs and geometric means of HLA-DR expression (r = 0.38, p = 0.03 and r = 0.36, p = 0.05, respectively; Pearson, 2 tailed significance).

Conclusions: In this study we show for the first time that DCs comprise a significant fraction of bone marrow derived cells in the FF. We speculate that in addition to their role at the implantation site of the endometrium, these cells actively contribute to the sterile inflammatory process in the follicle that ultimately leads to ovulation. In support of this notion, we now demonstrate a positive correlation between the ovarian response to gonadotropin stimulation and the presence of mature DCs within the follicle. The prognostic value of the follicular DC population warrants further investigation.

P-305 Over expression of luteinizing hormone receptors by stimulated human cumulus cells is in correlation with decreased fertilization rates

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Background: Luteinizing hormone (LH) triggers the maturation of the cumulus oocyte complex, which is followed by ovulation. Its action is mediated through the LH receptor (LHR). LH expression was investigated extensively in rodents, but hardly in humans.

Objective: We aimed to elucidate the LH expression patterns in human granulosa cells (GCs) from antral to preovulatory stages, and to investigate a correlation to oocyte function.

Approach: LH expression was examined in luteinized mural and cumulus GCs in different follicular developmental stages among women undergoing in vitro maturation (IVM) or in vitro fertilization (IVF) treatments. Luteinized preovulatory GCs were obtained from preovulatory follicles aspirated during IVF (≥ 17 mm). GCs from small- (<10 mm) and medium-sized (10-15 mm) follicles were obtained during IVM procedures. Cumulus GCs were obtained during oocyte denudation for intracytoplasmatic sperm injection procedures to determine LHR expression levels in mural and cumulus GCs of different follicular sizes and their correlation to oocyte outcome.

Results: LHR expression increased with follicle size and was higher in mural GCs compared to cumulus cells. LHR expression in cumulus GCs from preovulatory follicles was higher in metaphase II (MII) oocytes than in metaphase I or germinal vesicle oocytes (IVF). Unexpectedly, overexpression of LHR in cumulus GCs of MII oocytes correlated with decreased fertilization rates.

Conclusions: LHR expression in small follicles obtained in IVM suggests a role for human chorionic gonadotropin administration during IVM procedures. Overexpression of LHR in cumulus GCs of MII oocytes may signal malfunction of oocytes and low fertilization capacity.
Introduction: The well established function of the steroid hormone vitamin D is to maintain calcium and phosphorus homeostasis as well as promote bone mineralization. The discovery that most tissues and cells in the body have a vitamin D receptor and that several possess the enzymatic machinery to convert 25-hydroxyvitamin D, to the active form, 1,25-dihydroxyvitamin D, has provided new insights into the function of this vitamin. Among the many physiological processes influenced by this vitamin, its effects on reproductive physiology are investigated. Due to limited specific human data in this regard and realizing the epidemic of vitamin D deficiency in our country, in the setting of the roles of vitamin D in reproductive physiology; we conducted this study in order to evaluate the predictive value of follicular fluid vitamin D on assisted reproductive technique outcome in infertile patients referring to a tertiary academic center.

Material and Methods: A prospective cohort study was undertaken at Shariati Hospital. A total of eighty-two infertile women undergoing assisted reproductive technique were enrolled between August 2009 and March 2010. Women older than 38 years, those with systemic illness or consuming drugs interfering with vitamin D metabolism, hypothyroidism, amenorrhea, and women with galactorrhea were excluded from the study. Couples requiring biopsy for sperm recovery were excluded. Standardized regimens for pituitary down regulation and controlled ovarian superstimulation were employed. Serum samples (calcium, phosphorus, alkaline phosphatase, 25-hydroxy vitamin D and Parathyroid hormone) were collected on the day of ovum pick up. Follicular fluid (FF) for 25-hydroxy vitamin D (25OH-D) was collected from follicles ≥ 14mm; following oocyte isolation. After fertilization through intracytoplasmic sperm injection (ICSI), three good quality embryos were transferred transvaginally 3 days later. Pregnancy was detected by serum beta-hCG analysis 14 days after embryo transfer and transvaginal ultrasound scan was scheduled 2 weeks later to detect the pregnancy sac. Each pregnant woman was followed by ultrasound scan until fetal heart was documented (clinical pregnancy). The pregnant women were followed until 20 weeks of gestation (ongoing pregnancy).

Results: There was significant correlation between serum Vit D and FF Vit D (r = 0.767, p = 0.001). Serum Vit D was 20.3(13.4-34) nmol/l or 8.13(5.37-13.62) ng/ml in the clinically pregnant group and 21.45(14.80-53) nmol/l or 8.29(5.93-21.23) ng/ml in the non pregnant group (P = 0.235). Interestingly, follicular fluid Vit D was 22.95 (13.10-48.70) nmol/l or 9.19 (5.25-19.51) ng/ml in the clinically pregnant group and 25.80(17.40-74.10) nmol/l or 10.34 (5.89-29.69) ng/ml in the non pregnant group (P = 0.433). Although, fertilization rate was 71.5% in all the participants but fertilization rate in the first to third tertiles of FF 25OH-D was 79%, 67.8% and 68.3%, respectively (p = 0.018). Implantation rate was 12.6% in all women; but in the first to third tertiles of FF 25OH-D it was 9.6%, 13% and 15.9%, respectively (p = 0.791).

Conclusion(s): This study revealed that FF levels of 25OH-D are reflective of body stores of vitamin D. However; approximately all of our candidates were vitamin D deficient and we could not calculate the effect of vitamin D deficiency on ART outcome, but according to our ART results (36% chemical pregnancy and 30% clinical pregnancy), we may consider that vitamin D deficiency has not pivotal role on our ART results. On the other hand, higher reproduction rate observed in countries like Middle East countries which are among high prevalent vitamin D deficiency regions is not affected by male or female vitamin D reservoirs. Even though, this deficiency is prevalent among our pregnant women; then it may be considered that conceiving is not related to vitamin D stores.
Introduction: Symptomatic uterine leiomyomas can negatively affect the quality of life. Major surgery is the current main management option, while medical options are currently of more limited and mostly short-term use. Oral mifepristone, an antiprogestin, was shown to reduce uterine leiomyoma size and improve related symptoms. Vaginal mifepristone may be of greater usefulness as this route may offer rapid uptake, high local uterine levels and lower serum concentrations, bypassing liver metabolism with possibly less side effects.

Material and Methods: A total of 21 women, age 30-53, diagnosed with symptomatic uterine fibroids, received vaginally 10 mg of mifepristone daily during 3 months. Primary outcome was reduction in uterine leiomyoma volume, as assessed by ultrasonography at baseline, monthly during the course of therapy and at the final follow up three months after completing the study medications. Endometrial biopsies were obtained prior to, after one month and at end of treatment. Symptoms of uterine fibroids were assessed using the “Uterine Fibroid Symptoms Quality of Life Questionnaire” (UFS-QoL Score). Relevant Laboratory tests and bleeding patterns were recorded.

Results: Mifepristone treatment significantly reduced leiomyoma volume from a mean of 119.7 cc at enrollment to 89.4, 83.1, and 69.1 after 1, 2 and 3 months of treatment, respectively, with subsequent increase to 95.8 after 3 months without treatment (p < 0.05). The UFS-QoL Score significantly decreased (p < 0.01) from a mean ± SD of 202 ± 3.9 at enrollment to 173.2 ± 4.0, 14.2 ± 5.2, 13.9 ± 4.9 after 1, 2 and 3 months of treatment, respectively, and then increased to 15.5 ± 2.3 after 3 months without treatment. Mifepristone decreased the number of bleeding days. Endometrial biopsies showed no evidence of endometrial hyperplasia or cellular atypia.

Conclusions: This is the first study to show that vaginal mifepristone may offer an effective treatment option for women with symptomatic uterine leiomyoma and can improve the patients’ quality of life. Further study is needed to determine the long-term safety and efficacy, as well as the optimal dose and regimen of vaginal mifepristone, and its role as pretreatment for infertile patients with large leiomyomas seeking to improve their chances of conception.

P-311 Processing of semen from an HIV-1-positive male and its use in the IVF-ICSI procedure clinical efficacy

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While it has been documented that the density gradient/swim-up method substantially reduces the titers of HIV-1 in semen specimens obtained from infected males, approximately 5% of all prepared motile sperm fractions remain positive for HIV-1, and significant numbers of motile sperm are lost during the processing procedure. Furthermore, since even the highly sensitive HIV-1-detecting system sometimes yields false-positive results, whether the treatment should be continued or abandoned often poses a dilemma.

We have been performing HIV-1 elimination by sperm processing using a continuous density gradient, and examined the presence of HIV-1 in processed semen specimens and embryo culture medium by a highly sensitive PCR procedure. This study was conducted to examine the clinical efficacy of this procedure, based on the sperm recovery efficiency, results of HIV-1 positivity of the processed semen and also embryo culture medium, and the clinical results of IVF-ICSI.

Material and Methods: Two aliquots of semen specimens from the infected males were processed by centrifugation through a Percoll continuous density gradient (prepared by the tilted tube rotation method), and the recovered sperm fraction was underlaid beneath one ml of the culture medium. After 45 minutes incubation, the swim-up sperm was recovered from the upper 0.5-ml layer.

With written informed consent, the frozen-thawed sperm suspension that was negative for HIV-1 was used for ICSI with oocytes originating from non-HIV-1 infected wives. In each case, the resultant embryos after ICSI were cultured in two culture dishes, and the presence of HIV-1 in the embryo culture medium was examined in each of the culture dishes. Only the embryo from the culture dishes whose culture medium appeared to be HIV-negative were replaced. The lower limit of the PCR detection of HIV-1 was 50 copies/ml for both the sperm suspension and the culture medium.

Results: From January 2002 to December 2010, 334 semen specimens from 165 infected males were processed. The average sperm concentration and motility were 4803 ± 2423 x10^6/ml and 49 ± 21%. Motile sperm were recovered from 159 semen samples (96.3%), and specimens from both aliquots were HIV-1 negative in 154 (96.8%) cases. Nucleotide sequencing of the PCR product from 5 HIV-1-positive cases revealed a similar sequence to that of the blood-borne HIV-1 in the husband in 3 cases.

Two hundred thirty-three cycles of IVF-ICSI was performed for 140 couples. Forty hundred thirty embryo culture media specimens from 233 embryo replacement trials were examined for the presence of HIV-1 by PCR, and the culture media from both dishes were HIV-1 negative in 225 cases (96.6%). Among the 8 cases in which the presence of HIV-1 was suspected by PCR, the PCR product was sequenced in 5 specimens. The nucleotide sequences were similar to the sequence of the HIV-1 in the husband’s in one case, and similar to that of the positive control in 4 specimens.

The procedures have resulted in 54 pregnancies so far, including 47-singleton and 7-twin. No horizontal or vertical transmission occurred in any of the pregnant cases, and there was no case of horizontal transmission to the wives in any of the 70 non-pregnant cycles.

Conclusion: This method was not only effective for recovery of HIV-1-negative processed semen from HIV-1-positive individuals at a high probability, even from male-factor cases, but was also effective for detecting the residual HIV-1 gene in the processed semen specimens or embryo culture media. The clinical usefulness of this method was also confirmed by the birth of 61 babies without any evidence of horizontal or vertical transmission of HIV-1.

P-312 Non-equivalence of LH and hCG: an in vitro study

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Introduction: Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are heterodimeric glycoprotein hormones acting on the same receptor, the luteinizing hormone-chorionic gonadotropin receptor (LH/CGR). There are some hints that the two gonadotropins may have not the same activity at molecular, cellular and systemic level. Using recombinants LH and hCG, which can be quantified in molar terms, we aimed at investigating if the two gonadotropins have different ED50 value and leads to different kinetics of cAMP production over precise experimental time-periods.

Design: By using COS-7 cell line permanently expressing the human LH/CGR (COS-7/LH/CGR), we evaluated the LH and hCG effective dose at 50% of maximal response (ED50) by plotting dose-response curves drawn after measuring total cAMP accumulated over 3 hours incubation with different doses of LH or hCG in the pm-pM range, in the presence of the phosphodiesterase inhibitors 3-isobutyl-1-methylxanthine (IBMX). Also, the kinetics of cAMP stimulation by LH and hCG at equitope ED50 doses were compared in time-course experiments performed by measuring intracellular cAMP at different time-points over 3 hours of incubation with IBMX. Finally, the LH and hCG activity at ED50 doses was evaluated by performing 12-hours time-course experiments and by measuring intracellular cAMP in a physiological system, the human primary granulosa lutein cells (hGLC) naturally expressing the LH/CGR. The hGLC were used on the sixth day from ex vivo collection from women undergoing ovarian hyperstimulation for assisted reproduction to allow the recovery of the gonadotropic responsiveness after the clinical FSH stimulation, since sub-optimal response in the early days of culture was observed.

Results: In COS-7/LH/CGR cells, significantly different ED50 for LH and hCG were observed (LH: 475.75 ± 137.33 pm; hCG: 101.75 ± 44.63 pm, mean ± SD; Mann-Whitney’s U-test; p = 0.029; n = 4). Moreover, 3 hours
time-course experiments revealed that the maximal activation of intracellular cAMP reaches a plateau of 50-fold over control in just 10 minutes after LH stimulation, while the hCG stimulation results in maximal cAMP activation at the same level only after 1 hour, revealing a different kinetics of response (one-way Anova; p < 0.05; n = 3). Finally, the continuous exposure of hGLC to LH and hCG ED₉₀ dose for 12 hours reveals that the intracellular cAMP activation follows a repetitive and pulsatile rhythm with a frequency of about 3-4 hours and significantly higher levels of stimulation by hCG vs LH (one-way Anova; p < 0.05; n = 3).

Conclusions: Human recombinant LH and hCG have significantly different in vitro biopotency, since hCG stimulation results in an about 5-fold greater response than equimolar concentrations of LH. Moreover, equipotent concentrations (ED₉₀) of LH and hCG, resulted in a faster cAMP response to LH vs hCG, but a quantitatively higher response to hCG over 12 hours, revealing different kinetic of response mediated by the same, common receptor. Finally, the physiologic system hGLC responds to constant LH or hCG stimulation in a pulsatile fashion, with the same frequency but with different potency, suggesting a different novel control level of gonadotropic action at the receptor level.

P-313 The influence of dietary advice on assisted reproduction cycles outcomes

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Introduction: Adequate nutritional status is a critical determinant of the onset and maintenance of normal reproductive function. Nutritional deficits may represent a common contributing factor in the development of multiple neuroendocrine-metabolic disturbances underlying infertility. Moreover obesity in women has been shown to increase time to conception. Little data exist on the eating habits and body composition in women seeking infertility treatment. The goal for the present study was to evaluate the influence and the effectiveness of dietary advice in assisted reproduction cycles outcomes.

Material and Methods: The study included 60 patients undergoing controlled ovarian stimulation for intracytoplasmic sperm injection (ICSI) cycles in a private assisted reproduction center. In our center, all patients undergoing ICSI cycles may receive nutritional advices with no additional costs. Therefore, on the day of the embryo transfer, all patients filled a questionnaire containing multiple choice questions. The patients reported if they received nutritional advices or not, and among the patients that received nutritional advices, changes in eating habits were also reported. Patients that received nutritional advices were compared with those that did not receive or did not change its eating habits. Data were analyzed by student t test and logistic regression, adjusted for variables considered potential confounders of the association between the factors evaluated and ICSI outcomes (women age, dose of FSH administered, number of transferred embryos, infertility cause and endometrium thickness). Results were expressed as percentages, odds ratios (OR) with 95% confidence intervals (CI) and p-values. Results were considered to be significant at the 5% critical level (p < 0.05).

Results: Thirty two patients received nutritional advice and 28 did not receive nutritional advices or did not change its eating habits. The fertilisation (81.0% vs 67.1% P = 0.0225) and the pregnancy rates (46.9% vs 28.6% P = 0.0396) were significantly higher in the group of patients that changed its habits. This result was confirmed by the logistic regression model, which demonstrated more than twofold increase in pregnancy rate in patients who changed its dietary habits (OD: 2.27; CI: 0.63-8.15; P = 0.0408). Our results also showed that the pregnancy rate was positively correlated with the increased intake of whole bread (OD: 2.69; CI: 0.60-12.1; P = 0.0297), fruits (OD: 1.90; CI: 0.32-11.31; P = 0.0478) and vegetables (OD: 2.29; CI: 0.41-12.73; P = 0.0345).

Conclusions: Although the present study was conducted in a small number of patients, our data suggests that the professional dietary recommendation may be determinant of the likelihood of assisted reproduction treatments success. Indeed, patients that changed its eating habits according to the nutritionist advice had more than twofold increase in the successful pregnancy chance. Patients that followed the dietary recommendation increased the intake of whole food, fruits and vegetables, suggesting that the food composition, ie. minerals, essential aminoacids and antioxidant vitamins, influences the human metabolism, resulting in a better response to the assisted reproduction treatment. In conclusion our evidences suggest that professional dietary recommendation may be a valuable tool to increase the success rate in patients undergoing assisted reproduction treatments. However this study is to be continued to confirm our findings.

P-314 Are poor responders of advanced maternal age at higher risk for producing aneuploid embryos in vitro?

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Introduction: Poor responders’ management is one of the most difficult challenges to those carrying out assisted reproductive techniques (ART). Low follicular recruitment following ovarian stimulation may be observed in 9-24% of the ART cycles. Poor outcome after an initial low response cycle, regardless of the quality of embryos transferred or the age of the patient, suggests that embryos of good morphology may still have a low pregnancy potential in this group of patients. In addition, poor responder patients appear to be at greater risk for fetal aneuploidy compared to normoresponder patients. Despite the well established relationship between advanced age and diminished ovarian reserve, it is still unknown if, considering only patients with advanced maternal age, there is a difference between the incidences of chromosomally abnormal embryos when compared with a normoresponder women. The aim of this study was to chromosomally compare embryos originated from aged women according to ovarian response to stimulation.

Material and Methods: Using our center’s computerized database we retrospectively identified cycles of couples who underwent their first in vitro fertilization (IVF) treatment in conjunction with Preimplantation Genetic Screening (PGS), as a result of advanced maternal age. A total of 84 ICSI cycles were identified and split into two groups according to the number of retrieved oocyte following ovarian stimulation: Normoresponder (NR group, N = 50), patients who produced more than four oocytes; and Poor Responder (PR group, N = 34), patients in which up to four oocytes were retrieved. Embryos reaching at least the 6-cell stage on day 3 of development were biopsied and a two-round FISH procedure was performed, which allowed for the detection of chromosomes X, Y, 13, 15, 16, 18, 21 and 22. Embryo transfer was performed on day 5. Influence of poor ovarian response on aneuploidy rates was assessed using logistic regression analysis, and results are expressed as odds ratio (OR), confidence intervals (CI) and p-values. Results were considered to be significant at the 5% critical level (p < 0.05).

Results: A total of 485 embryos were successfully biopsied. There were no significant differences between NR and PR groups regarding the percentage of aneuploid embryos (51.0% [n = 213] vs 58.2% [n = 39], p = 0.270), embryos showing autosomal aneuploidy (45.9% [n = 192] vs 50.7% [n = 34], p = 0.2659), sexual aneuploidy (15.1% [n = 63] vs 14.9% [n = 10], p = 0.4355) and multiple abnormalities (21.1% [n = 88] vs 26.8% [n = 18], p = 0.2368). Logistic regression analyses showed no influence of poor response on the incidence of embryo aneuploidy (OR: 1.33, CI: 0.65 - 2.75, p = 0.271), embryo sexual aneuploidy (OR: 0.9, CI: 0.32 - 2.50, p = 0.836) and autosomal aneuploidy (OR: 1.23, CI: 1.23 - 2.53, p = 0.566). However, poor response was determinant
of the likelihood of embryo multiple abnormalities occurrence (OR: 2.15, CI: 0.88 – 5.25, p = 0.0017). Although fertilization (83.9% vs 87.1%, p = 0.6861), clinical pregnancy (28.0% vs 29.4%, p = 0.9208), implantation rates (22.6% vs 20.6%, p = 0.6863) and miscarriage rates (8.3% vs 7.7%, p = 0.6788) were not significantly different, the percentage of cycles without embryo transfer (4.0% [n = 2] vs 23.5% [n = 8], p = 0.0128) was significantly higher on PR group. This finding was confirmed using a binary logistic regression, showing that poor response was determinant of the likelihood of embryo transfer occurrence (OR: 7.38, CI: 2.35 – 2.24, p = 0.016). Therefore, the mean number of embryos transferred (2.0 ± 0.9 vs 1.1 ± 0.8, p = 0.0001) was significantly lower on PR group.

**Conclusion:** In conclusion, aged females responding poorly to gonadotrophins are not at a higher risk for producing aneuploidy embryos in vitro. Therefore, poor ovarian response to gonadotrophins does not justify the implementation of PGS in these patient’s cycles due to the association with a statistically reduced number of cycles transferred and a lower number of embryos transferred per patient.

**P-315 Carbohydrate metabolism and mitochondrial activity by human follicles and oocytes throughout early folliculogenesis and oogenesis**

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**Introduction:** The characterisation of follicle and oocyte carbohydrate metabolism and mitochondrial activity throughout folliculogenesis and oogenesis is of value for the optimisation of follicle and oocyte culture conditions, the improvement of assisted reproduction technologies (ART) and the identification of non-invasive markers of oocyte quality. Glucose and pyruvate are of key importance for oocyte, follicle and embryo development as they are the main source of energy. Carbohydrate metabolism by mammalian preimplantation embryos, preovulatory follicles and fully grown oocytes has been well documented, and embryo glucose and pyruvate metabolism has been related to embryo developmental competence. However, little is known about the early stages of follicle and oocyte development. Studies in mice suggest that follicle and oocyte metabolism changes as development and maturation progress. To date there is no data on energy metabolism in early human folliculogenesis and oogenesis. Therefore, this study aimed to quantify glucose and pyruvate consumption, lactate production and mitochondrial activity by human follicles and oocytes from primordial to secondary stages of follicle development.

**Material and Methods:** Carbohydrate metabolism was quantified in oocytes and follicles harvested from cryopreserved human ovarian cortex. Ovarian cortex samples were donated for research by 7 patients aged 30.7 ± 1.8 years. The tissue was cryopreserved by slow cooling in 1.5M DMSO and 0.1M sucrose and stored for 11.4 ± 0.4 years. After thawing, primordial to secondary follicles were harvested by needle isolation following digestion in Collagenase Type IA (740U/ml) and DNase (8.4U/ml). Primary oocytes were denuded by exposure to trypsin (0.6mg/ml). Viable follicles and oocytes, as confirmed by neutral red (NR) staining, were cultured individually or in groups in 31nl or 95nl drops of spent culture media was measured using ultramicrofluorescence assays. The ratio of active vs. inactive mitochondria was analysed following JC1 staining of NR stained samples were donated for research by 7 patients aged 30.7 ± 3.4 years. Mitochondrial activity was measured following JC1 staining.

Viability, assessed by NR staining, had no impact (p > 0.05) upon follicle metabolism.

There was a trend towards oocyte pyruvate consumption increasing (p = 0.055) from primordial (0.3 ± 0.04 pmoles/oocyte/h; n = 5) through primary (0.8 ± 0.1 pmoles/oocyte/h; n = 8) to secondary follicles (1.3 pmoles/oocyte/h; n = 3). Pyruvate consumption rate per unit of oocyte volume was lower (p < 0.005) in non viable –compared to viable- oocytes at primordial to primary stages. Oocyte mitochondrial activity was highly variable both within and between stages of oocyte development, and ranged from 14.2 ± 1.5 arbitrary units (min = 8.7, max = 17.2; n = 5) in primordial oocytes to 13.6 ± 7.9 arbitrary units (min = 1.2, max = 28.4, n = 3) in oocytes from primary follicles; or 5.8 arbitrary units (n = 1) in oocytes from secondary follicles.

**Conclusions:** This is the first report of direct measurement of carbohydrate turnover and mitochondrial activity in the early stages of human folliculogenesis and oogenesis. This study provides insight into how the metabolic requirements of follicles and oocytes change as development progresses and suggests that these parameters may be altered by culture conditions and some ARTs such as cryopreservation.

**P-316 First trimester screening for Down syndrome in assisted conceptions according to the use of fresh or frozen embryos**

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**Introduction:** The influence of embryo freezing on first trimester Down syndrome screening in pregnancies achieved by assisted reproduction techniques has not been determined. The aim of the present study is to compare the screening between pregnancies achieved by IVF-ICSI according to the type of embryo transferred (fresh versus frozen).

**Material and Methods:** Retrospective study. Comparison of nuchal translucency (NT), free β fraction of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) MoM (multiple of median) values, as well as false positive (FP) screening tests, in normal singleton pregnancies conceived by assisted conception using fresh (N = 1537) or frozen (N = 312) embryos. The same comparison was also performed considering the origin of the oocytes- fresh (N = 971) versus frozen (N = 194) in native oocytes; and fresh (N = 563) versus frozen (N = 103) in donated oocytes- and the mode of insecumation –fresh versus frozen with IVF alone (N = 96 vs N = 34 in native oocytes; and N = 192 vs N = 41 in ovum donation) or ICSI alone (N = 811 vs N = 145 in native oocytes; and N = 353 vs N = 60 in ovum donation).

**Results:** No significant differences were detected in any of the comparisons performed. However, in both groups of fresh and frozen embryos the FP Down syndrome screening rate tended to be higher when native oocytes were employed and especially in the ICSI group, although without statistical differences. In fact, FP rates were 8.3% with fresh embryos and 8.2% with frozen embryos in native ICSI cycles.

**Conclusions:** This study suggests that first trimester Down syndrome screening has a similar reliability in pregnancies achieved by assisted reproduction regardless the type of embryo transferred (fresh versus frozen).

**P-317 Oocyte collection results with the use of a novel quasi double lumen needle which enables aspiration and flushing through the same single lumen**

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**Introduction:** Double lumen needles with one lumen connected to an aspiration pump and the other connected to a flushing pump or syringe, have been used for oocyte collection in IVF. Whether flushing increases number of oocytes collected is controversial but the procedure absolutely takes longer with patient discomfort and ovarian trauma, while providing an effective lumen size.

An ideal needle should have the smallest possible outer diameter to decrease patient discomfort and ovarian trauma, while providing an effective lumen size. Perhaps oocyte recovery rate can be improved without compromising the flow rate and substantially increasing operating time.
We report the use of a novel quasi double lumen needle, which enables aspiration and flushing through the same single lumen. 

**Material and Methods:** The quasi double lumen needle is a 18 G single lumen needle with an external diameter of 1.2 mm and an internal diameter of 0.82 mm (corresponds to 21G). The tail of the needle is connected to an aspiration pump as any single lumen needle. Starting from the 7th centimeter from the needle tip, the needle shaft is covered with a thin plastic tubing, which is connected to an irrigation pump, similar to a double lumen needle. 8 cm from the needle tip there are two holes drilled into the needle which connect the needle lumen with the potential space between the outer surface of the needle and the inner surface of the plastic tubing. follicular fluid is initially aspirated through the 21 G lumen. When the aspiration pump is stopped and the flushing pump is activated, irrigation medium travels through the space between the needle and the sheath, flows into the needle lumen through the two holes and reach follicular cavity. When flushing is stopped and aspiration pressure is re-activated the flushing medium is again aspirated through the single lumen. The 7 centimeters long needle tip allows the operator to reach the follicles without inserting plastic covered needle shaft through the vaginal vault.

31 women underwent oocyte collection using the new needle following controlled ovarian hyperstimulation. Total of 543 follicles sized were punctured. Follicles were flushed up to 4 times if an oocyte was not identified in the aspirate. Total of 339 oocytes were recovered resulting in 62.4% recovery rate per follicle. 44.8% (152/339) of the collected oocytes were identified in the initial aspirate before flushing. 29.1% (158/543) were identified after flushing twice, 5.3% (29/339) after flushing four times.

**Conclusion:** The new quasi double lumen needle enabled follicular aspiration and flushing in all cases. Although the overall oocyte recovery rate of 62.4% can be considered moderate, it should be noted that the calculation is not limited to larger follicles but includes follicles as small as 9 – 10 mm. Oocyte recovery rate of 44.8% in the initial aspirate can be considered less than satisfactory. Therefore increasing needle gauge from 18 G to 17 G which corresponds to an outer diameter of 1.47 mm, and an inner diameter 1.06 mm may required. In IVM the flushing technique can be used in needles down to 22G avoiding painful and long acting repeated reinserstion of needle into ovaries. The result indicate, that flushing up to two times can increase number of collected oocytes, whereas extensive flushing only marginally increase egg retrieval rates.

**P-318 Effect of granulocyte colony-stimulating factor on pregnancy outcome following IVF/ICSI in patients with repeated implantation failure**

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**Introduction:** This study was performed to evaluate the effect of granulocyte colony-stimulating factor (G-CSF) on pregnancy outcome following IVF/ICSI in patients with repeated implantation failure (RIF).

**Material and Methods:** Eighty-two patients with RIF, aged 29-40 years, were recruited for this prospective study. RIF was defined as the failure of good quality embryos (grade I or II) to implant after at least 3 cycles of IVF/ICSI in infertile women without thrombophilia and anatomic abnormalities of uterine cavity. They were randomly assigned to G-CSF group (n = 41) or control group (n = 41). GnRH antagonist multiple dose protocol (MDP) using recombinant human FSH (rFSH) was used for controlled ovarian stimulation (COS) in all subjects. Embryo transfer (ET) was performed 3 days after oocyte retrieval. For G-CSF group, recombinant human G-CSF at a dose of 100 mcg was administered on the day of ET and the fourth day after ET.

**Results:** G-CSF group and control group were comparable with respect to the age of patients, body mass index (BMI), infertility duration, endocrine profile and indications for IVF/ICSI. COS and IVF results were also comparable between the two groups Clinical pregnancy rate and embryo implantation rate were significantly higher in G-CSF group than those in control group (48.8% vs 26.8%, P = 0.040, 22.1% vs 9.1%, P = 0.004, respectively). In singleton pregnant women following IVF/ICSI, serum hCG level on the eleventh day after ET was significantly higher in G-CSF group of 171.6 ± 52.6 mIU/mL compared with 81.9 ± 31.7 mIU/mL in control group (P < 0.001).

**Conclusions:** G-CSF treatment can improve the pregnancy outcome of IVF/ICSI in patients with RIF, and may enhance the development of trophoblast.

**P-319 Description of the telomeric RNA, TERRA, in human oocytes**

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**Introduction:** The ends of lineal chromosomes are organized in ribonucleoprotein complexes called telomeres, essential to maintain genome integrity. Furthermore, in oogenesis telomeres play an important role throughout meiotic chromosome events since they tether telomeres together promoting alignment, synopsis and recombination between homologous chromosomes. The recently discovered telomeric repeat-containing RNA (TERRA) is a structural component of the telomeric complex that also acts as a telomerase inhibitor. Until now, TERRA has been described in several somatic cell lines and tissues of different eukaryotic organisms. In this study, TERRA was identified for the first time at telomeres of human oocytes, and its presence and localization was established all through the first stages of meiotic prophase I.

**Material and Methods:** For this study 229 human oocytes from ovaries of two patients aged 29-32 years were used. The ovarian tissue was collected on slides through mechanical disgregation, and then cells were permeabilized with CSK buffer. At this point, both synaptonemal complex protein 3 (SYCP3) and telomeric repeat-binding factor 2 (TRF2) were immunostained enabling discrimination of prophase I oocytes among ovarian tissue cells, identification of prophase I stages, as well as localization of telomeres at chromosome ends. Afterwards, TERRA was labeled with an oligonucleotide probe (CCCTAAA), by the RNA-Fluorescence In Situ Hybridization (RNA-FISH) technique.

**Results:** The combined use of immunofluorescence and RNA-FISH techniques allowed us to observe both TERRA and proteins SYCP3 and TRF2 in all fetal ovaries’ cell types, revealing that TERRA co-localizes with TRF2 at telomeres, forming discrete foci at both oocytes and the rest of ovarian tissue cells. About 60-70% of the analyzed oocytes were TERRA-positive from leptotene to pachytene stages, and it was statistically verified that the presence/absence of TERRA was not subject to the prophase I stage. Similarly, clustering of chromosomes when arranged in the bouquet structure did not influence the ratio of oocytes showing TERRA at their telomeres.

Then, when determining TERRA foci throughout prophase I progression, statistically significant differences were observed, suggesting that the number of TERRA foci per cell is subject to the prophase stage. Actually, at both zygote and pachytene TERRA levels remain similar, while at leptotene differences among oocytes are much more noticeable. However, statistical analysis showed that the relationship between TERRA levels and the prophase stage is nevertheless very weak; indicating that these differences might disappear with a higher number of analyzed oocytes. Finally, oocytes showing bouquet structure do not display significant differences, and its rates are similar to those observed at zygote and pachytene.

**Conclusions:** In this study, a technique combining immunofluorescence and RNA-FISH was optimized, allowing qualitative analysis of TERRA in human fetal ovarian tissue. TERRA forms discrete foci at telomeres of the whole cell types of ovarian tissue, co-localizing with the shelterin component TRF2. Moreover, TERRA is present all through prophase I, from leptotene to pachytene stages, and also when chromosomes perform the bouquet structure. Eventually, TERRA levels seem to remain steady throughout meiotic prophase I. Overall, our results open a new area for the study of oogenesis’ development and regulation in women.

**P-320 Gene expression in human fetal oocyte is altered by bispfenol A exposure**

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This study was performed to evaluate the effect of bispfenol A on gene expression in human fetal oocytes.
Introduction: Bisphenol A (BPA) is a “weak” endocrine disruptor. The effect of BPA on human reproduction is controversial but has been related to meiotic anomalies, recurrent miscarriages and abnormal karyotypes. BPA crosses the placenta and recent studies have reported high levels in placental tissues and amniotic liquid. A previous report in *C elegans* showed that BPA induces sterility, reducing the number of viable oocytes and embryo as well as impaired chromosome synapsis, disruption of double-strand breaks (DBS) repair progression and impaired chromosome segregation during metaphase. Studies performed in exposed mice have shown synaptic defects like end-to-end chromosome associations and incomplete synapsis, increased levels of MLH1 foci number and chiasmata. Exposed human fetal oocytes have shown a diminution of oocyte survival, delay of meiotic progression and an elevated rate of MLH1 foci number in vitro. BPA’s effects on gene expression had been studied in different tissues and cells; in ovary, only one study has shown alterations after BPA exposure in mouse. Mice exposed to BPA showed significant up-regulation of transcripts implied in meiotic pairing-synthesis genes.

The aim of this study is to characterize the gene expression of BPA-exposed and unexposed oocytes cultured human fetal ovaries. Evaluated transcripts were related to cohesion proteins (SMCβ), synapsis proteins (SCY1p), DNA-DBS generation, signaling and reparation (SP01, RPA, HA2X, MLH1 and BLM), estrogen receptors (ERα, ERβ and ERRγ) and markers of meiotic progression (STRA8 and NALP5).

Material and Methods: To accomplish our objective, 12 ovaries from euploid fetuses were used. The ovarian fetal tissue was cultivated following the technique described by Briëno et al. (2010). The tissue was cultivated with serum free D-MEM medium (supplemented with SCF 90ng/ml and ITC) and divided into two groups: Control-group and BPA-group (culture medium + BPA30μM). The cultures were analyzed at T0 (fresh tissue), T7, T14 and T21. Gene expression evaluation was performed by real-time PCR (RT-PCR). Total RNA was obtained using the RNeasy fibrous tissue kit (Qiagen) following the manufacturer’s instructions. Quantitative RT-PCR amplification was performed in a Bio-Rad CFX-Real time PCR detection system. The ribosomal sub-unit 18S gene was used as an endogenous control. Each reaction mixture consisted of cDNA and PCR mix containing forward and reverse primers, SYBR-Premix Ex Taq (TaKaRa) and nuclease-free water. PCR conditions were established for each gene.

Results: Gene expression was evaluated in oocytes cultured with control-group and no differences were observed among different times of culture for SMCβ, SCY1p, SP01, RPA, HA2X, MLH1, BLM, ERα, ERβ and ERRγ. STRA8 showed an increment of gene expression at T7 and T14 followed by a diminution at T21 corresponding to the progression of oogonias into oocytes. NALP5 showed an increment of gene expression at T14 and T21 corresponding to the progression of oocytes into primordial follicles. BPA did not affect gene expression of SMCβ and SCY1p. BPA up-regulated gene expression of genes implied in DNA-DBS generation, signaling and reparation. SP01, HA2X and BLM genes showed an increment of 3 to 5-fold excess in gene expression, as compared to control (p ≤ 0.05) and RPA 100-fold excess (p ≤ 0.01). Surprisingly, gene expression of MLH1 was not affected by BPA. Estrogen receptors ERα, ERβ and ERRγ showed a BPA up-regulation in all of the culture times the increment of gene expression was from a 2 to 4-fold excess compared to control (p ≤ 0.05).

Conclusions: Gene expression of oocytes from control-group did not showed differences, as compared to oocytes in fresh. Oocytes exposed to BPA showed an up-regulation of genes implied in DNA-DBS generation, signaling and reparation as well as estrogen receptors. BPA did not showed alterations in the gene expression of genes implied in cohesion and meiotic pairing-synthesis progression.

P-312 Systemic and follicular oxidative stress and antioxidant status in IVF patients

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Introduction: Several endogenously synthesized reactive oxygen species (ROS) play an important role in the cellular signaling events leading to cell growth, differentiation, migration, mitosis and other processes. Total antioxidant status (TAS) regulates the levels of different ROS to maintain their optimal physiological low level as their excess can lead to high-grade oxidative stress (OS), causing aberrations in DNA, proteins and lipids, and in the long term being deleterious for the cell or tissue. It has been proposed that the expression of antioxidant system components in complex organisms varies between tissues and the response to environmental OS factors may be tissue- and organ-specific. A positive outcome in *in vitro* fertilization (IVF) procedure requires the successful occurrence of several events: from folliculogenesis, oocyte maturation and fertilization up to embryo implantation and normal development. Therefore, the levels of both systemic (measured in blood or urine) and intra-follicular OS and TAS are potential indicators for the outcome of IVF. In the current study, our goal was to describe the significance of OS and TAS in the oocyte maturation environment as well as systemically in IVF female patients and correlate the results with the outcome of ovarian follicle stimulation, clinical pregnancy rate and etiology of infertility.

Material and Methods: Blood plasma, urine samples and follicular fluid (FF) were collected from 102 IVF patients undergoing controlled ovarian stimulation according to the GnRH antagonist protocol and ICSI treatment. Total peroxide concentration (TPX) was chosen as a marker of OS and determined by ferrous oxidation in xylene orange assay. TAS was measured via the total antioxidant response (TAR) method based on the capacity of antioxidants in a sample to suppress Fe3+/−-dianisidine reduction by H2O2. Both TPX and TAR were determined in FF and blood. The oxidative stress index (OSI) was calculated as the percentage ratio of TPX to TAR reflecting the overall balance of OS and TAS in the sample. The urinary content of 8-iso-prostaglandin F2α (8-isoPs), an end-product of lipid peroxidation and a widely accepted additional marker of systemic OS, was measured in the urine samples using commercial competitive ELISA (BIOXYTECH® 8-F-ISOs Assay, OxisResearch®, Portland, OR, USA).

Results: The values of TAR were significantly lower and those of OSI significantly higher in FF compared with blood plasma samples. Only TAR values showed statistically significant positive correlation between plasma and FF. Follicular TPX and OSI positively associated with blood estradiol on the day of follicle puncture and the number of oocytes obtained, while OSI negatively correlated with the dose of follicle-stimulating hormone used per oocyte retrieved. High urinary 8-isoPs related to lower embryo quality and was elevated in smoking patients (p < 0.05 for all mentioned correlations). A tendency towards higher systemic blood TAR was observed in patients achieving clinical pregnancy (p = 0.052), while endometriosis patients demonstrated reduced follicular TAR (p = 0.01).

Conclusions: We clearly show for the first time that follicular OS markers correlate to stimulation efficiency and that the redox environment is completely different between blood plasma and follicular fluid in IVF patients. We could demonstrate that reduced intra-follicular TAS is a characteristic of endometriosis patients and that achievement of clinical pregnancy is influenced by systemic TAS. Our work clearly reveals the association of both, systemic and local OS and TAS markers, with various aspects of IVF outcome.

P-322 Lipopolysaccharide activates bovine granulosa cells via TLR4 and perturbs oocyte meiotic progression in vitro

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Introduction: Infection of the female genital tract with Gram-negative bacteria commonly causes pelvic inflammatory disease (PID) and infertility in women and cattle. Ovarian follicle development and function is perturbed in animals with PID, and lipopolysaccharide (LPS) from Gram-negative bacteria is readily isolated from the follicular fluid of women and cattle with PID. Immune cells mount an inflammatory response to LPS when it is recognised by Toll-like receptor 4 (TLR4), which is a central component of the innate...
immune system. Using the cow as a model, we hypothesise that LPS found in follicular fluid can initiate an inflammatory response by TLR4 on granulosa cells, altering the follicular environment and ultimately perturb the meiotic competence of oocytes.

**Material and Methods:** Granulosa cells and cumulus-oocyte complexes (COCs) were isolated from healthy cow ovaries following aspiration of 4–8 mm follicles. Granulosa cells were challenged with a range of pathogen associated molecular patterns (PAMPs) to determine their ability to signal through various TLRs and initiate an inflammatory response. Granulosa cells were incubated in the presence of 10-fold increasing concentrations of ultrapure LPS (TLR4 ligand), lipoteichoic acid (LTA; TLR2 ligand), peptidoglycan (PGN; TLR2 ligand) or Pam3CSK4 (PAM; TLR1 and 2 ligand) ranging between 100pg/ml and 10μg/ml. The pro-inflammatory cytokine IL-6 and the chemokine IL-8 were measured in cell-free supernatants by ELISA. Acute responses to LPS were evaluated by qPCR for IL6 and IL8, while the phosphorylation of ERK 1/2 and p38-MAPK was assessed by immunoblot. TLR4 specific siRNA was used to demonstrate the importance of TLR4 in LPS-initiated inflammation.

In vitro maturation (IVM) of intact COCs was performed using defined media in the presence of LPS. Subsequently, meiotic progression of oocytes was assessed by confocal microscopy preceded by immuno-histochemistry to label meiotic spindle structures. Oocytes which failed to reach the M-phase of meiosis II, or those which had significantly perturbed meiotic structures following IVM were deemed to have failed meiosis.

**Results:** Granulosa cells accumulated IL-6 and IL-8 in a dose dependent manner in response to LPS, LTA, PGN and PAM compared to untreated controls (P < 0.05). The accumulation of IL-6 and IL-8 was further increased following 48h of culture for each PAMP (P < 0.05). IL-6 and IL-8 mRNA was significantly increased in cells following only 30 min exposure to 1 μg/ml of LPS compared to untreated controls (2.1- and 2.8-fold, respectively) and remained elevated at 180 min (2.5- and 5.3-fold, respectively; P < 0.05). Untreated cells showed little or no initial phosphorylation of p38-MAPK or ERK1/2, however following a 30 min treatment with 1μg/ml of LPS, phosphorylation of both ERK 1/2 and p38-MAPK was increased and maintained up to 180 min. After 24 h transfection with TLR4-siRNA granulosa cells had a 52.4% reduction in accumulation of IL-6 in response to 24 h LPS exposure compared to untreated controls (P < 0.05). LPS increased the expansion rate of COCs in the absence of FSH (0% vs 24%, P < 0.05), possibly due to increased IL-6 production compared to untreated controls (4.1-fold increase, P < 0.05). Meiotic progression of 290 COCs was assessed to ascertain the impact of LPS exposure. Untreated COCs had a meiotic failure rate of only 14.0%, however, the addition of 10μg/ml of LPS significantly increased the meiotic failure rate to 34.4% (P < 0.05). Meiotic progression of 290 COCs was assessed to ascertain the impact of LPS exposure. Untreated COCs had a meiotic failure rate of only 14.0%, however, the addition of 10μg/ml of LPS significantly increased the meiotic failure rate to 34.4% (P < 0.05).

**Conclusions:** For the first time these data indicate that bovine granulosa cells initiate an inflammatory response to LPS via the TLR4 pathway. Consequently, perturbations to the follicular environment adversely affect the developing oocyte and its meiotic competence. We suggest that reproductive tract infections negatively impact the developing cohort of oocytes.

**P-323** **NK cells in IVF treated patients**

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**Introduction:** Decreasing fertility of endemic population represents a serious socio-economic problem in almost all developed countries and elicits an increasing effort in the diagnosis and treatment of fertility disorders. Much credit has been given for the technique of in vitro fertilization with subsequent transfer of the embryo (IVF-ET). Unfortunately, the success rate of this method is limited by low implantation rate (slightly over 30%). Consequently, the attention of investigators now switches from the technique of IVF towards the questions of embryo implantation. It follows from the very nature of implantation that the crucial factor of maternal-fetal interaction is immunity. Approximately 70% of lymphocytes residing in the pregnant decidua are NK cells. These are phenotypically distinct from peripheral blood NK cells in expression of extremely high levels of CD56. Proposed functions of NK cells in the gravid (nebo pregnant?) uterus are: production of cytokines to facilitate decidualization and control of the invading trophoblast protection of fetal tissues from maternal immune attack and protection of the fetus from infectious diseases.

**Methods:** We analyzed the peripheral blood and the follicular fluid of patients from IVF center of the Institute for the Care of Mother and Child, Prague. The cohort consists of patients prepared for IVF-ET. Using FACS assay we compared successful vs unsuccessful IVF patients’ cell populations: CD56dim/CD16- (NK – cytokine producing cells) CD56dim/CD16+ (NK – cytotoxic cells) and CD161/NKG-2D expression (activation of these cells) along with standard CD3/CD4/CD8 panel for T cells. We focused our attention on KIR2DL4 and CD85J (ILT2, LIR) expression, because these markers are associated with functional attenuation.

**Results and Discussion:** We observed increased surface expression (mean fluorescence intensity) of KIR2DL4 on cytotoxic NK cells (CD56dim/CD16+) in follicular fluids of successfully fertilized patients. Increased KIR2DL4 expression on these cells was also observed in their activated variants (CD56dim/CD16+/CD161+/NKG-2D+). Peripheral blood exerted increased CD85J surface expression on T helper cells (CD3+CD4+CD8+), bearing NK cell activation marker NKG-2D in the case of successfully fertilized patients.

**Conclusions:** These results indicate the important role of KIR2DL4 mediated attenuation of NK cell function in the scope of IVF success rate. The higher CD85J (ILT2 or LIR) expression on T helper cells may have contributed to overall immune attenuation and IVF success.