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FC 1: Counselling, ethics & moral issues

FC 1-1 Ethics and moral issues
Survey of 125 former sperm donors

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For decades, in sperm banks, university laboratories and doctors’ offices all over the world, a valuable commodity has been regularly deposited. It has always been unregulated and secretive. And it is sold at a price to desperate couples, single women and same-sex partners willing to do almost anything to conceive a child. Although a genetic connection between the mother and child was acknowledged as important, the genetic connection between the child and its genetic father was seen as non-existent.

This study presents findings from a large sample of sperm donors recruited by the Donor Sibling Registry, a U.S.-based non-profit organization that helps to connect, educate and supply support for donor-conceived children, parents and donors. An online survey asked about medical and health issues, contact with clinics, reasons for donating, contact with offspring, donors’ satisfaction with the donation process, and current feelings.

Results

Results were based on 125 sperm donors. 22.5% report donating at more than one facility, with 32.1% of those having donated at 3 or more facilities and 3 donors accepted at 15 or more clinics. 83.8% have never been contacted by their clinic to update their medical information, although 25.7% do say that they have had health concerns or genetic risk factors that they believe would be of interest to recipient families. 94.2% of donars say they would have been willing to receive genetic testing at the time of donation, yet no sperm banks ask their donors to submit to any such testing. 79.8% report that they did not receive any education or counselling on the curiosities of genetic children is lacking as well as medical follow ups by the clinics. Donors are open to and are establishing meaningful relationships with their offspring.

Conclusions

Our findings reveal sperm donor’s thoughts and feelings about their donations, some decades after donating. There is a need for clinics and sperm banks to give donors clearer guidelines regarding donating at multiple clinics and to giving the clinic medical updates to benefit their genetic children. Proper counselling and education pre-donation about probable curiosities of genetic children is lacking as well as medical follow ups by the clinics. Donors are open to and are establishing meaningful relationships with their offspring.

FC 1-2 Counselling
Counselling guidelines for cross-border reproductive care

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There are indications that the number of patients travelling abroad for assisted reproductive treatment (ART) is increasing. The reasons for cross-border reproductive care (CBRC) are manifold and include accessing treatments legally prohibited or rejected for moral reasons in a patient’s home country as well as benefiting from higher quality treatment and/or from lower costs. CBRC is challenging not only from a medical and ethical perspective but also from a psychological one. Infertility counselling, in several countries considered to be both an integral part of ART and crucial in third party conception, is rarely provided in countries typically frequently for CBRC.

Regardless of one’s own opinion of CBRC, patients are confronted with a multitude of challenges. Language difficulties as well as notoriously unreliable information and advertisements on the internet make informed decisions difficult. Patients require comprehensive and unbiased information and counselling in order to appraise treatment options, success rates and potential risks. For many patients, CBRC is perceived to be their last chance to fulfil their wish for a child. This can lead to severe distress and anxiety, making them vulnerable to emotional and commercial exploitation. In order to provide high-quality psychosocial care and counselling, the German Society for Fertility Counselling has developed „Counselling Guidelines for Cross-Border Reproductive Care“. Psychosocial counselling prior to CBRC is non-directive; its primary aim is to empower patients to make autonomous decisions for or against treatment abroad. These guidelines highlight the importance of
• the provision of unbiased basic medical and legal information (esp. in the case of surrogacy)
• provision of information about the risks of multiples
• the exploration of and understanding for couple and family dynamics after third party reproduction and surrogacy
• the need to consider the best interest of the child, including the child’s need to have access to his/her biological origin
• consideration of the welfare of the oocyte donor, the semen donor and the surrogate as well as consideration of her/his meaning for and role in the family-to-be.

The development of professional care standards and their integration into professional practice is one step towards increasing patients’ autonomous decision-making as well as demonstrating responsible practice.

FC 1-3 Ethics and moral issues
Information provision and decision-making in ART. Results from a national survey in Germany

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Background

In a national survey, we investigated how patients (P), reproductive physicians (RP), and psychosocial counselors (PC) in Germany assess information provision in fertility care, conceive the burdens of treatment, report the incidence of overwhelming desires for children, and judge the patients’ competence and authority to make treatment decisions.

Methods

Standard questionnaire techniques (paper and pencil, computer-aided web interviewing).

Results

We received a total of 638 questionnaires from RP (response rate RR = 36%), 99 from PC (RR = 66%) and 1590 from P (RR = n. d.). The absolute majority of P (60–68%) felt generally well informed by their reproductive physicians; 64% of the RP saw generally little problems with providing information to their patients, while 38% of the PC assessed patients’ overall level of information as good. With regard to a number of specific aspects, between 11% and 65% of
the P stated that they received good or very good information (mean: 41%). The percentage of RP and PC who assessed the patients’ level of information as good or very good varied between 8% and 59% (mean: 25%) and 2–53% (mean: 14%), respectively. Emotional burdens of the treatment were perceived as high or very high by 85% of the P. Three quarters (74%) of them affirmed that they have experienced an overwhelmingly strong desire for a child causing other purposes of life to recede into the background. Among RP and PC, 33% and 42% confirmed that they encountered such patients often, respectively. Almost half (47%) of the P reported the feeling of losing control over the situation. According to 25% of the RP and 47% of the PC, patients are often or very often limited in their competence to decide when to stop the treatment. The majority in all study populations (50–69%) granted patients the final decision to have further treatment when prospects are low.

Conclusions The results provide evidence that patients are often not well informed about relevant aspects of their treatment. At the same time, there is evidence that many patients are overwhelmed by their longing for a child, have lost control over the situation and have limited competence in finding an end of the treatment if it is not successful. Patient counselling should be improved in order to strengthen their decision-making competence and to avoid harmful treatments. The indication for treatment should be more tightly controlled by physicians.

FC 1-4 Counselling
The effect of infertility counselling on the quality of marital relationship in infertile couples

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Background Infertility is a major factor that leads to sexual and marital dissatisfaction. In many couples, the infertility crisis can be seen as a cumulative trauma, which indicates that these couples have a marked need for infertility counseling. The purpose of this study was to determine the effect of counseling on infertile couple’s sexual and marital satisfaction that referred to the fertility center in Tehran, Iran.

Methods The 100 infertile couples were recruited in this study by convenience sampling method and then randomly allocated to two groups (50 couples in counselling and 50 couples in control groups). Study was designed in two phases pretest before intervention and was followed up 3 months later. Counselling group participated during 3 hours through 3 sessions every week. Data was collected by 3 demographic, marital and sexual satisfaction questionnaires. Data were analyzed with SPSS software and Chi², Mann Whitney and Wilcoxon test (p < 0.05).

Results There was a significant difference between sexual satisfaction in women of 2 groups 3 months after intervention (u = 741.500; p = 0.019).

The research findings revealed that there is a significant difference between sexual satisfaction in the men of 2 groups 3 months after intervention (u = 746.500; p = 0.02). Considerable difference in the marital satisfaction between women in 2 groups 3 months after intervention by Man Whitney test obtained (u = 776.000; p = 0.036). There was a significant difference in marital satisfaction in the men between 2 groups 3 months after intervention (u = 756.500; p = 0.022).

Conclusion The results of this study supported that counselling increased the sexual and marital satisfaction of infertile couples.

FC 1-5 Counselling
Motivations, experiences and attitudes of Argentineans’ anonymous oocyte donors

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Objective Studies about oocyte donors show that, in general, women are altruistically motivated to donate oocytes but most would not donate them if a financial compensation is not offered. Thus, the selection of oocyte donors is very important to achieve good pregnancy outcomes. The objective of the present study is to evaluate the reasons that motivated a group of Argentines anonymous oocyte donors towards oocyte donation.

Materials and Methods 115 women applied for donation. The inclusion criteria were: being healthy fertile women between 21 and 31 years old, have proven fertility and an appropriate gonadal function. The donors completed a detailed written application form which included personal and familial health history, obstetric and gynaecologic history, educational history and work history. They underwent rigorous clinical evaluation (hormonal levels, physical characteristics, karyotype, cystic fibrosis, sexually transmitted diseases, and use of drugs) and psychological evaluation. The donors were followed up with 45 minutes in-depth psychological interviews during the evaluation, the ovulation induction and at the end of the procedure. Donors were asked about their motivations to donate with an opened question: ‘What do you feel is your main motivation to donate oocytes?’ The procedures were approved by the IRB and all donors signed informed consent forms prior to the donation process.

Results 23 out of 115 potential donors were selected (20%). The mean age was 25 years. All were Caucasian with 1 to 5 children. Marital status: 91.3% single and 8.7% married. Moreover 39.1% lived with their partners, 60.9% lived with their partners. Additionally, 25.4% were married. Moreover 39.1% lived with their partners, 60.9% lived with their partners. Moreover 39.1% lived with their partners, 60.9% lived with their partners.

Conclusions Financial compensation and research purposes received the same level of motivation. However the higher rate of donors answered that helping infertile women was their primary motivation to donate oocytes. It is very interesting to analyze the strength of empathy awakened towards infertile women. In the present group of donors their main motivation was altruistic.

FC 1-6 Counselling
Embryo donation: A follow-up analysis of donor experiences

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Background Patients who have completed IVF treatment often have excess embryos. In Victoria, the options available include donation to other patients, research or disposal. Previous studies reveal donation of surplus embryos to others remains consistently low, despite research indicating patient support for donation is high. However in some of these studies, patients with excess embryos in storage were yet to make a decision regarding their fate. The literature suggests decision-making about surplus embryos is complex and subject to change.

Aim This retrospective study evaluates the short and long term experiences of embryo donors, to gauge influences in decision-making and post donation and assess overall post-decision satisfaction.

Study Population and Methods Of patients considering the fate of excess embryos, 6% choose donation; such couples donating embryos between 1988 and 2008 were sent a questionnaire in 2009 asking about their experiences prior to and post donation. Likert scales recorded participants’ responses to a series of statements on a continuum of agreement/disagreement. The questionnaire evaluated three areas of decision-making: Satisfaction- Uncertainty, Informed Choice and Decision Control.

Results 480 questionnaires were posted, 230 returned. Most participants (63%) found the decision to donate a relatively easy one, however 37% reported difficulty or experienced mixed feelings. Overall half of respondents (58%), said decision-making had been emotionally charged for them and 67% reported satisfaction with the amount of information received to inform decision-making.
FC 2: Pre-implantation embryo & cryopreservation

Effect of ovarian tissue cryopreservation on the meiotic competence of the murine oocyte

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Introduction Due to improvements in chemo- and radiotherapy, the number of young cancer survivors is increasing. One long-term side effect of these therapies is the premature loss of ovarian function in female patients. In order to maintain the patients’ ability to conceive children after successful cancer treatment, new fertility preservation strategies are required. The aim of this study was to investigate the effect of ovarian tissue cryopreservation on oocyte meiotic competence in a mouse model.

Materials and Methods Ovaries were collected from 7- to 17-week-old female C57Bl/6 mice (n = 9) and cryopreserved following a slow freezing protocol using DMSO, ethylene glycol and sucrose as cryoprotectants. After thawing, oocytes were mechanically isolated with 27G needles and in vitro maturation (IVM) of oocytes was performed for 16–20 hrs under administration of 1.5 mM/L H and 1.5 mM/L FSH. Subsequently, oocytes were classified using an inverted microscope according to their meiotic stage as germinal vesicle (GV), germinal vesicle breakdown (GVBD) and metaphase II (MII). Oocytes isolated from non-cryopreserved ovaries from comparable mice served as controls.

Results The number of GV oocytes resuming meiotic development was similar be-
between the two groups. After cryopreservation, 79 out of 90 oocytes (88%) resumed meiosis, compared to 114 out of 124 (92%) oocytes, which matured after isolation from fresh tissue, without previous stimulation- or superovulation-treatment of the animals.

Conclusions Isolation and IVM of murine oocytes derived from cryopreserved ovarian tissue was successfully performed and showed no major difference in meiotic re- sumption compared to controls. Further in- vestigations evaluating fertilization rates of these in vitro matured oocytes need to be performed.

FC 2-2 Pre-implantation embryo Comparison of elective cryopreservation of embryos at the pronucleate stage and blastocyst stage in the women at high risk of OHSS

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Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening condition which can occur if oocytes are excessively stimulated during IVF treatment. Patients have their embryo transfer cancelled and em- bryos cryopreserved. This study compared outcomes of embryos cryopreserved at pronucleate stage versus vitrification at blastocyst stage for patients at risk of OHSS.

Over a period of 3 years (2006-2009) em- bryo transfer was cancelled for 86 cycles. 54 of these cycles utilised slow-controlled freezing of pronucleate embryos and, to date, 435 embryos have been thawed for blastocyst culture and transfer. 22.3% (97/435) of the thawed embryos developed into morulae or blastocysts and were transferred. In the other 32 cycles all embryos were cultured to blastocyst prior to freezing. Of these 49% (198/408) embryos were vitrified after success- ful blastocyst formation. 71 of vitrified embryos have been thawed for blastocyst prior to freezing. Of these 49% (198/408) embryos were vitrified after successful blastocyst formation.

In the group where pronucleate stage em- bryos were thawed, 16.88% (13/77) of cycles were cancelled due to lack of blastocyst forma- tion after 5 days of culture. In comparison, 78.87% (56/71) of warmed blastocysts survived, and just 1.92% (1/52) cycles were can- celled. Clinical pregnancy rate resulting from warmed blastocysts was 27.45% and 24.74% respectively from the other 32 cycles all embryos were cultured to blastocyst prior to freezing. Of these 49% (198/408) embryos were vitrified after success- ful blastocyst formation.

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Preliminary results indicate no significant difference in pregnancy and implantation rates. However the proportion of embryos which formed blastocysts suitable for transfer was lower in the pronucleate group. In addition, the lower cancellation from warming vitrified blastocysts reduced patient stress and frustration and improved treatment cost effectiveness.

This research is privately funded.

FC 2-3 Cryopreservation Cryopreservation and subsequent xenografting of Marmoset ovarian tissue into immunodeficient mice: Effects of different cryoprotective agents on follicular survival and grafting success

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Introduction Recent improvements in cancer survival rates have brought along a re- newed interest in cryopreservation of ovarian tissue for fertility preservation. Xenografting of cryopreserved ovarian tissue can demon- strate follicular viability and developmental competence. We used the common marmoset as a non-human primate model to assess the effect of different cryoprotectives on follicu- lar viability of adult and prepubertal ovarian tissue following xenografting.

Materials and Methods Animals: Ovarian tissues from adult and pre- pubertal female marmosets. Cryopreservation: Tissue fragments were cryopreserved with dimethylsulfoxide (DMSO, 1.5 mol/L), 1,2-propanediol (PrOH, 1.5 mol/L) or ethylenglycol (EG, 0.5 mol/L) using a slow freezing protocol.

Surgical procedures: Ovariectomized and int- act nude mice (Crl:NU-Foxn1nu) received 8 grafts per animal. The tissue was placed in subcutaneous pouches in the dorsal skin and retrieved after 2, 4 and 8 weeks.

Histological evaluation: The tissue was rou- tinely fixed, embedded and serially sectioned (5 µm); every 5th section was evaluated for number of follicles in each class (primordial, primary, advanced) and viability of follicles.

Immunohistochemistry: Positive staining for PCNA demonstrated follicular viability.

Results Following cryopreservation, the relative number of morphologically normal primordial follicles in adult tissues decreased significantly from 46.45 ± 4.6 to 12.28 ± 1.7 (DMSO), 9.55 ± 1.7 (PrOH) or 6.83 ± 1.0 (EG), respectively. After xenografting, the relative number of morphologically normal primordial and primary follicles in the DMSO group was significantly higher (26.2 ± 2.5 and 28.07 ± 5.4) than in the PrOH group (12.17 ± 3.5 and 5.42 ± 2.1). PCNA staining suggests the initiation of growth in all cellular compartments of xenografted tissue. Primordial follicles in prepubertal tis- sue display a similar sensitivity to the cryoprotectant (DMSO: 28.22 ± 1.6, PrOH: 11.67 ± 2.2, EG: 14.35 ± 1.6). Unlike to adult tissues, there are no significant differences between DMSO and PrOH following xenografting.

Discussion DMSO is superior compared with ProOH and EG. In prepubertal tissue, significant differences between DMSO and PrOH were abrogated following xenografting, probably owing to a greater capacity of younger individuals to compensate for cryo- injury.

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FC 2-4 Pre-implantation embryo Seminal fluid provokes expansion of Regulatory T cells during early murine pregnancy

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Regulatory T cells (Treg) expand very early in pregnancy and are thought to be major contributors to the acquisition and mainte- nance of tolerance towards the fetus. In mice, Treg were shown to be antigen-specific and to expand upon paternal antigens; however the nature of antigens supporting their expansion is quite unknown. First contact between paternal antigens and the maternal im- mune system takes place at insemination. Thus, we aimed to investigate participation of paternal sperm or antigens from semi- nal fluid as factors provoking the expansion of Treg at the beginning of pregnancy.

We mated CBA/J female mice with intact Balb/c males, vasectomized Balb/c (lacking sperm) or seminal vesicles deficient Balb/c (lacking seminal fluid). On pregnancy days 0.5; 2 or 5 females were sacrificed and per- centage of Treg was determined in thymus, lymph nodes, only the absence of seminal fluid as factors provoking the expansion of Treg at the beginning of pregnancy.

We showed that the presence of seminal fluid or seminal vesicles deficiency provoked a significant increase in the frequency of Treg (expansion was significantly impaired when paternal antigens were missing). Therefore we aimed to investigate provoking the expansion of paternal antigens as factors provoking the expansion of Treg at the beginning of pregnancy.

We observed an expansion of Treg in CBA/J females after mating with intact Balb/c as compared to virgin controls. In thymus this expansion was significantly impaired when sperm and seminal fluid were missing. In lymph nodes, only the absence of seminal fluid prevented Treg expansion. In vitro we showed that the presence of seminal fluid obtained from Balb/c males provoked a sig- nificant increase in the frequency of Treg from CBA/J females which was abolished after blocking TGF-β1. Culture of Treg from Balb/c females with seminal fluid showed only slight effects on Treg expansion.
Introduction Cryopreservation of human semen may cause damages to the spermatozoa, probably due to the production of reactive oxygen species. The aim of this prospective study was to analyze oxidative stress markers and seminal standard parameters before and after using an important antioxidant, the Resveratrol.

Materials and Methods Ejaculated spermatozoa from 20 infertile men and 10 fertile donors with proven fertility were prospectively examined. Oxidative stress markers including the levels of thiobarbituric acid reactive species (TBARS; lipid peroxidation product) and the activity of the antioxidant enzymes superoxide dismutase (Sod) and catalase (Cat) were evaluated. Semen samples were divided into 4 aliquots which were treated as follows: no resveratrol, 0.1 mM, 1.0 mM, and 10.0 mM of Resveratrol. All 4 aliquots were divided into 20% cryopreservation and sperm motility, SOD, Cat and TBARS were evaluated both before cryopreservation and after thawing. Results were analyzed by ANOVA and Tukey’s post hoc test. Person correlation was used to calculate the relationship between response variables.

Results Cryopreservation causes a decrease in Sod activity in fertile men, both with or without the use of resveratrol (p = 0.03). However, Cat activity was higher in all post-thawing samples, both in fertile and infertile men, when compared to the pre-freezing samples (p = 0.04). The addition of resveratrol was able to increase Sod activity irrespectively of its concentration in the post-thawing samples of infertile men (p = 0.03). Both fertile and infertile men showed increased TBARS values after cryopreservation, which were prevented by resveratrol, irrespective of its concentration (p = 0.03). A negative correlation was found between sperm motility and TBARS values in infertile men, both in the pre-freezing (-0.868; p < 0.05) and in the post-thawing (-0.897; p < 0.05) samples.

Conclusions Resveratrol was able to prevent post-thawing lipid peroxidation both in fertile and infertile men, reflected by the increase on TBARS and improve on antioxidant activity. However, this antioxidant was not able to prevent the motility decrease caused by cryopreservation. The present study showed that resveratrol is able to minimize lipid peroxidative damages and that it could be considered in human cryopreservation procedures, both for fertile and infertile men, at least for intracytoplasmic sperm injection, as it does not need motile sperm for achieving pregnancy.

Funding none.

FC 2-5 Cryopreservation

In vitro addition of resveratrol during cryopreservation prevents sperm damage in fertile and infertile men

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Introduction Cryopreservation of human semen may cause damages to the spermatozoa, probably due to the production of reactive oxygen species. The aim of this prospective study was to analyze oxidative stress markers and seminal standard parameters before and after using an important antioxidant, the Resveratrol.

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Conclusions Resveratrol was able to prevent post-thawing lipid peroxidation both in fertile and infertile men, reflected by the increase on TBARS and improve on antioxidant activity. However, this antioxidant was not able to prevent the motility decrease caused by cryopreservation. The present study showed that resveratrol is able to minimize lipid peroxidative damages and that it could be considered in human cryopreservation procedures, both for fertile and infertile men, at least for intracytoplasmic sperm injection, as it does not need motile sperm for achieving pregnancy.

Funding none.

FC 2-6 Cryopreservation

Efficient vitrification of oocytes and zygotes under reduced cooling conditions in a hermetically sealed straw

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Introduction Vitrification of oocytes and zygotes with “open” devices, where there is a direct contact of the biological material with liquid nitrogen (LN2), bears the risk of contamination. To minimize this risk, “closed” devices for the vitrification of blastocysts were successfully introduced. However due to thermo-isolation of “closed” carriers, which induces a remarkable drop in the cooling rate (< 2,000 c/ min.), a certain fear has been reported for aseptic vitrification of large cells such as oocytes and zygotes that seems more sensitive to reduced cooling rate.

We report our results on the vitrification of oocytes and zygotes in a “closed” system that eliminates the potential danger of contamination. Before cooling, the MII oocytes and zygotes were gradually exposed to a final solution of 20%/20% DMSO/ethyleneglycol. They were then placed on the aseptic gutter of a “Vitrisafe” straw which was inserted in an outer protective straw. Welding of the protective straw, ensuring a hermetic isolation of the sample took place before plunging into LN2. For warming the tip of the gutter containing the oocytes or zygotes was directly immersed into 1M sucrose and they were transferred to droplets with decreasing sucrose concentration before culture until day 3 or 5. A total of 146 oocytes were vitrified and warmed (22 cycles). From the 137 patients who were administered GnRH agonist: group 1 consisted of the cycles in which blastocysts had been derived from patients who were administered GnRH agonist (n = 1,087), while group 2 consisted of the cycles in which blastocysts had been derived from patients who were administered GnRH antagonist (n = 997). All embryos developed to the expanded blastocyst stage on day 5 or 6 were vitrified following artificial shrinkage. The solution for vitrification was composed of DPBS containing 20% (v/v) HFF, 40% (v/v) ethyleneglycol, 18% (w/v) Ficoll, and 0.3 M sucrose. Warming of the vitrified blastocysts was carried out in 2 steps on day 3 after ovulation [Lee et al., 2006]. The thawed blastocysts were cultured overnight and then transferred the next day. The implantation and ongoing pregnancy rates were compared between the 2 groups.

Results The number of cycles which underwent controlled ovarian hyperstimulation (COH) by GnRH agonist, followed by the transfer of fresh embryos, was 4,722 (33.7 ± 3.5 yrs), while the number of cycles which underwent COH by GnRH antagonist, followed by the transfer of fresh embryos, was 5,011 (34.4 ± 4.1 yrs). The clinical preg-
nancy and blastocyst vitrification rates of the former were 36.4% and 49.0%, respectively, which were higher than those of latter (28.2 and 33.2%, respectively). However, there was no difference in the survival rate between groups 1 and 2 (89.5 vs 90.7%). The number of vitrified-warmed blastocyst stage embryos that were transferred was similar in groups 1 and 2 (2.4 ± 0.8 vs 2.5 ± 0.8). The ongoing pregnancy and implantation rates in group 2 were significantly higher than those in group 1 (37.7 vs 30.5%; 23.6 vs 18.4%, respectively) (p < 0.001).

**Conclusion** These results suggest that use of GnRH antagonist for ovulation induction could be detrimental to endometrium, but the resulting blastocyst stage embryos might be more viable comparable to those from GnRH agonist.

**FC 2-8 Pre-implantation embryo What have we learned from PGD?**

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**Aims** A relatively high percentage of embryos generated in IVF are chromosomally abnormal (Munné et al. 1995; Magli et al., 2001; Munné et al., 2007). A correlation has been reported between embryo development and chromosomal abnormalities, with a higher rate of embryo abnormalities being seen in embryos with abnormal cell division and morphology (Magli et al., 2007). The aim of this study was to determine whether embryo selection for transfer based only on morphological criteria correlates with normal chromosomal composition.

**Methods** A retrospective double-blind study with 69 IVF-PGD cycles was carried out. A total of 136 embryos were analyzed by FISH for chromosomes X, Y, 13, 15, 16, 17, 18, 21, 22 (Vysis). In order to test our objective, an examiner blinded to the PGD results made a selection of two embryos per cycle for a hypothetical transfer on day 3 of embryo development using strict morphological criteria. Morphological criteria for embryo screening included number of cells, blastomere symmetry, multinucleation and fragmentation. An embryo scoring system from 1 to 10 was established, with 10 being assigned to embryos with normal chromosomal composition.

**Results** PGD analysis showed that 47.9% of high-quality embryos (scored as 10, 9 or 8) and 70.4% of medium-quality embryos (scored as 7, 6 or 5) had chromosomal abnormalities. If strict morphological criteria had been used for embryo selection, only 14.4% of the transfers would have resulted in the transfer of two chromosomally normal embryos, 60.8% would have resulted in the transfer of one chromosomally abnormal embryo and 21.7% in the transfer of two chromosomally abnormal embryos. Should those potentially transferable (but chromosomally abnormal) embryos have been transferred, then 21.7% would have had chromosomal abnormalities compatible with a live birth and 78.3% would have had chromosomal abnormalities not compatible with a live birth.

**Conclusions** The results show that, based on morphological criteria:

- 47.9% of high-quality embryos have chromosomal abnormalities and that this percentage increases as embryo morphology worsens;
- only 14% of the hypothetical transfers would have resulted in the transfer of 2 chromosomally normal embryos; and
- only 21.7% of the potentially transferable embryos would have had chromosomal abnormalities compatible with a live birth.

This study highlights the importance of not just using morphological criteria when selecting embryos for transfer in couples with genetic risk.

**FC 3: Reproductive endocrinology I**

**FC 3-1 Reproductive endocrinology Preliminary findings from a new subspeciality reproductive medicine clinical module for medical students**

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**Background** These data describe medical student examination results and course feedback for a clinical and laboratory training module in reproductive endocrinology and infertility (REI).

**Methods** We surveyed upper-level medical students (n = 66) who attended lectures at Sims IVF as part of their RCSi obstetrics and gynaecology rotation at Coombe Womens & Infants University Hospital. Medical students had REI lectures, observational sessions in the embryology laboratory, and time was also allocated to discuss research projects and publications. Data were obtained from 2008–2009 students via anonymous, voluntary questionnaires collected at the end of the rotation.

**Results** Written examination was mandatory for all students and some feedback on course quality was provided by all students. Fully completed questionnaires were returned by 42/66 (63.6% response rate). Average (± SD) student age was 24.3 ± 2.3 yrs (range = 21–29), 77.3% female and 22.7% male. UK and Irish nationals comprised only 2.4% and 16.7% of students, respectively. In our sample, 33% of medical students had a graduate degree while only 4.5% had any prior formal exposure to REI. Mean final examination score for the REI module was 78.9 ± 11 (range = 45–95). Most students (61.9%) considered REI as a very important topic during medical school, and more than half (57.2%) acknowledged their baseline knowledge as inadequate. All student learning objectives in REI were met in an “above average” manner in self-reports by 92.9% of respondents.

**Conclusions** Information from this study suggests that a structured clinical and laboratory training module as implemented here is viewed favourably by medical students, and should help optimise educational experiences for REI in this setting.

**FC 3-2 Contraception Voluntary pregnancy interruption and following contraception choice**

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**Objective** Our retrospective study analyses the post-abortive contraceptive choices of women who underwent voluntary pregnancy interruption (VPI) in our Clinic.

**Methods** We collected data about women who underwent surgical VPI during 2008 in the Clinic of Obstetrics and Gynecology of the University Hospital of Udine. Data was analyzed by R (version 2.10.0) considering significant p < 0.05.

**Results** The mean age of women undergoing VPI is 29.6 years (SD ± 6.76). Inside this group there is a higher incidence of immigrants women 51.0% than in the general population (11.0%) (p < 0.05). The unemployment prevalence in the VPI population is 43.0% where in the general population of our region is 4.4% (p < 0.05). The majority of pluripara is represented by immigrants from Asia 92.0%, Subsaharian Africa 61.0%, and Eastern Europe 73.0%, and are significantly more prevalent than Italian pluripara 49.0% (p < 0.05). After VPI, 16.9% of women decided to assume oral contraceptives (OC) and 18.1% to insert a copper IUD, while 39.8% remained sceptic and 25.3% denied any possibility of contraception other than barrier or natural methods. In the group of women who had already had a previous VPI there was a higher prevalence of a clear contraceptive decision (60.0%) than in the general population (26.9%) (p < 0.05). There was a higher use of IUD in case of recurrent VPI and multipara, and a wider use of OC by first and nullipara (p < 0.05). If compared to nullipara (17.1%), primipara (43.8%) and pluripara (28.0%) had a higher prevalence of indecision about post-VPI contraception.

**Conclusions** There is still a lot to do in order to REI knowledge in adequate post-VPI contraception, and in particular to provide a better explanation about low cost contraception in women who have undergone VPI. There is still a lot to do in order to REI knowledge in adequate post-VPI contraception, and in particular to provide a better explanation about low cost contraception in women who have undergone VPI.
the low income groups, using a multilanguage advertising for the new immigrant people.

**FC 3-3 Contraception**

**Preventing pregnancy and sexually transmitted disease infections, by novel and unique approach**

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**Objectives**

- To provide women with an alternative to the condom that is covert, female-controlled and coitaly-independent.
- To kill sperm and STI/HIV organisms upon deposition into the vagina, and before they enter the cervix.
- To shield the cervix from sperm penetration, STI/HIV invasion and microbicides irritation.

**Design and Methods**

Women are the fastest growing demographic of STI/HIV infections worldwide. Ulcerative and non-ulcerative STIs enhance the transmission of HIV. Though the condom offers the best prevention against STI/HIV, the condom is not an option for many women. Thus far the condom and microbicides have failed to impact the spread of these infections. As these old and familiar methods repeatedly fail, we strongly believe that STI/HIV prevention must be tackled from a different angle. Therefore, we urgently need an alternative to the condom that is covert, woman-controlled, and deliver microbicide on the vaginal side. The FemCap is a new cervical barrier contraceptive device that is available in all European countries and US. The FemCap is designed to shield the cervix, (site of CCR-5 and CXCR-4 receptors), from microbicide irritation, STI/HIV invasion and sperm penetration. The FemCap delivers the microbicide on the vaginal side, to encounter sperm and STI/HIV organisms upon deposition into the vagina and before they enter the cervix. Ten women applied a microbicide mixed with Gentamicin and dexamethasone on the vaginal side of the FemCap, and inserted it vaginally. The cervix and vagina were photographed before, during, and 6 hours after removal of the FemCap.

**Results**

The bulk of the microbicide/dye came out upon removal of the FemCap without staining the cervix, while the vaginal walls were lightly stained with the dye.

**Conclusions**

This dual strategy of a FemCap with microbicde can prevent pregnancy and potentially STI/HIV infections and promote safer sex without decreasing its pleasurable sensations. The FemCap/microbicde combination may provide women with an alternative to the condom that is covert, woman-controlled and coitaly-independent. The FemCap/microbicde warrant further comparative head-to-head research with the condom to prove this concept.

**FC 3-4 Reproductive endocrinology**

**Oral nimodipine, a lipid soluble calcium channel blocker, inhibits the ovarian cycle in mice**

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**Introduction**

Nimodipine, is a lipid soluble calcium channel blocker that has a great affinity for the cerebral vasculature due its ability to cross the blood-brain barrier. It is used in a clinical setting to reduce the severity of neurological deficits in patients who have had a recent subarachnoid hemorrhage. The intrinsic pulsatile secretion of gonadotropin releasing hormone (GnRH) has been directly associated with the rhythmic changes of intracellular calcium concentration in GnRH neurons. On a cellular level, nimodipine has been shown to inhibit the pulsatile activity of GnRH gene expression in GnRH neurons. We therefore postulated that nimodipine, will inhibit the LH surge that is necessary for spontaneous ovulation and completion of the ovarian cycle.

**Materials and Methods**

We analyzed estrus cycles of female ICR mice (n = 24) treated with Nimodipine (Sigma) or vehicle. The completion of the estrus cycle indicated the presence of an LH surge. Vaginal gross appearance confirmed by cytology was used to determine the stages of the estrus cycle: diestrus, pro-estrus, estrus and meta-estrus.

**Results**

Our preliminary results indicated that daily intraperitoneal injections of nimodipine (5 mg/kg/day) were effective in preventing the completion of the estrus cycle in the mice. However, a more convenient mode of administration we utilized was oral gavage. We adjusted the dose to 15 and 25 mg/kg/day to account for the first pass effect caused by metabolism in the liver.

We performed 2 independent experiments in which the estrus cycles of a total of 24 mice were monitored. After reaching diestrus, 12 mice were treated with oral Nimodipine (15 mg/kg/d, n = 6; 25 mg/kg/d, n = 6) and 12 control mice were treated with vehicle. 8 of 12 control mice completed the estrus cycle as compared to only 2 of the 12 nimodipine treated mice (p = 0.0036; 2 sided Fisher’s Exact test). Notably, the two mice that completed estrus cycle in the treated group were both in the low dose group (15 mg/kg/d). Importantly, the inhibition of the completion of the estrus cycle was achieved without signs of toxicity or weight loss in the treated animals.

**Conclusions**

Our mouse study demonstrates that oral nimodipine inhibits the ovarian cycle in a dose related manner. The potential use of this inexpensive oral medication as an alternative strategy to GnRH agonists or GnRH antagonists to suppress the LH surge during assisted reproduction warrants further investigations.

**FC 3-5 Contraception**

A comparative analysis of high and low contraceptive use in Africa: Evidence from DHS

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The aim of this article is to show a comparative analysis of contraceptive use in areas of traditionally high fertility has gone through profound changes. This study analyses the contraceptive use in several developing countries in Africa and highlights the unsatisified needs of contraception, to understand why such needs exist. Data has been taken from the latest Demographic and Health Surveys (DHS). Analysis in the form of logistic regression model by users for four selected representative countries: Egypt, Namibia, Mali and Niger based on 2 criteria: data should be recent, and selected countries should have high (Egypt [57.4%] and Namibia [46.4%]) and low (Mali [7.5%] and Niger [10.0%]) contraceptive use, respectively. Contraception data is always gathered at a point of time, but cross-sectional data is not sufficient to understand all the mechanisms hidden behind contraceptive use. Different contraceptive behaviours that need good estimation tools to develop specific family planning programmes.

**FC 3-6 Reproductive endocrinology**

Abnormal circadian rhythm of salivary cortisol is closely related with ovarian dysfunction and ART pregnancy loss: Importance of stress-response-mechanism

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**Introduction**

Stress causes infertility and ovarian dysfunction. Stress response is controlled by hypothalamic-pituitary-adrenocortical (HPA) axis and sympathoadrenal system (SAS). We analyzed relation of HPA axis and SAS with various types of ovarian dysfunction and IVF outcomes.

**Methods**

In study 1, a total of 157 women with ovarian dysfunction based on four basal body temperature and 7 normal volunteers were studied. The patients were classified to 8 groups: PCOS, 49 women; PCOS with hysteral abortion, 7; low body weight, 10; elevated FSH, 24; elevated PRL, 6; endometriosis, 10; high age, 4; and idiopathic, 47. HPA axis was analyzed by the circadian rhythm of salivary cortisol and dexameth-
sone suppression test (DST) on cycle days 3–7. The circadian rhythm was classified into normal (N) type and 4 abnormal types; low-morning (LM), plateau (P), V-shaped (V), and flat (F) types. SAS was analyzed by measuring plasma catecholamines. In study 2, the rates of pregnancy and abortion were compared between patients with normal and abnormal HPA axis in 178 IVF cycles.

**Results**

**Study 1:** The incidence of abnormal circadian rhythm in salivary cortisol was significantly higher in women with ovarian dysfunction than in normal volunteers (48% vs. 0%). The incidence was high in PCOS with habitual abortion (86%), low body weight (60%) and PCOS (53%), and moderate in elevated PRL (50%), idiopathic (47%) and elevated FSH (42%), and low in endometriosis (30%) and high age (0%). DST was worsened in types N, LM, P, V and F in this order. The incidence of elevated catecholamines was prominently high in PCOS with habitual abortion (86%), compared with other groups of ovarian dysfunction (0–27%) and normal volunteers (0%).

**Study 2:** IVF pregnancy rates were similar in N, LM, P, V and F types, but abortion rate was significantly higher in V and F types than in N, LM and P types (50% vs 19%). Abortion rate was significantly higher with abnormal DST than with normal DST (50% vs 21.4%).

**Conclusions**

Abnormal HPA axis was identified by the circadian rhythm of salivary cortisol in half of women with ovarian dysfunction, especially more frequently in PCOS and low body weight. Abnormal HPA axis and SAS were prominently frequent in PCOS with habitual abortion, and also were associated with abortion in IVF. Stress-response-mechanism is closely related to ovarian function and maintenance of pregnancy, and its normalization may work as a novel treatment of infertility and pregnancy loss.

**FC 3-7 Contraception**

**Evaluation of reversible contraceptive efficacy in male rats of 3 herbal plants used for cancer treatment**

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**Objective** Today population explosion is a serious threat to mankind existence. Fertility control is the only means to resolve this global concern since uncontrolled population poses a barrier to sustainable development. Abnormal or varying types of contraceptives has been used to control population but cause some side-effects in users. Historically numerous herbs have been used to reduce fertility in human being and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested. Fertility based on the simplified formula: $T = T + k \int [\Omega \text{Est} - d \text{GnRH/GnRH}_d]$

**Results**

1. The hormonal armamentarium of the female reproductive systems ensures single ovulation and single pregnancy.
2. Unlike bone marrow ovaries do not possess a stochastic mechanism and rely solely on suppression of nearby follicles by the dominant follicle.
3. Hypothalamus senses the risk of MP and adjusts pulsation of GnRH in the consecutive cycle by prolonging the low and postponing the high frequency pulsation. Like electric circuits, external stimuli have a changing strength of 1/4 to 1/15 meaning that they can advance or retard ovulation in this range (percentage of the innate cycle of that person).
4. The strongest predictor and determining parameter affecting menstruation cycle is sensing the MP risk by the hypothalamus exemplifying itself as frequency of GnRH pulsation (dGnRH/GnRH dt).
5. Menstruation cycle and fertility can be predicted by mathematical formulas of parametric oscillation.

**FC 4-1 Reproductive endocrinology**

**Different expression pattern of chemokine and angiogenic factors during proteoglycan Syndecan-1 knock-down in decidualized human endometrial stroma cells**

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Embryonic implantation depends on an orchestrated early fetal-maternal dialogue. Chemokines belong to those early factors and play essential roles in the maternal reproductive tract leading to morphological changes upon decidualization, mediating maternal acceptance towards the semi-allograft embryo and induction of angiogenesis. Chemokine ligand 1 (CXCL1) was shown to be expressed in the decidua after contact with embryonic secretion products. The binding of chemokines to their G-protein coupled receptors is supported by the syndecan (Sdc) family of heparin and chondroitin sulfate proteoglycans. Syndecans are localized on the cell-surface and in the extra-cellular matrix. They consist of an ectodomain containing consensus sequences for heparan sulfate or chondroitin sulfate attachment, a single conserved transmembrane domain and a short cytoplasmic domain. They are described as multifunctional molecules in...
human, localized nearly ubiquitously and being involved in wound healing, tumor growth, immune cell function and angiogenesis. Sdc-1 acts as a chemokine co-receptor and is involved in chemokine storage and regulation of signaling cascades. Furthermore, it is supposed to interact with angiogenic factors in human endometrial stromal fibroblasts upon decidualization. Sdc-1 was shown to be expressed stronger in secretory versus proliferative phase endometrium during the window of implantation and it was also found in invading chorionic villi, suggesting a role in the fetal-maternal dialogue.

The aim of the present study was to characterize the role of Sdc-1 regarding chemokine and angiogenic factor expression at the fetal-maternal interface during the peri-implantation period in the human endometrium. A stable and inducible Sdc-1 knock-down was generated in the immortalized human endometrial stroma cell line St-T1. Dot blot analyses of decidualized knock-down cells versus non-transfected controls, displayed significant changes in cytokine and angiogenic factor expression profiles on protein level regarding inflammation, matrix regulation and angiogenesis of Sdc-1 knock-down cells. Therefore, we conclude that Sdc-1 seems to play an important role as a co-receptor and storage factor for many cytokines and angiogenic factors during decidualization supporting a regular implantation and angiogenesis.

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FC 4-2 Reproductive endocrinology

Efficacy and outcomes of assisted reproductive technologies programmes in women with thyroid autoimmunity

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Objectives To assess efficacy and outcomes of assisted reproductive technologies (ART) programmes in euthyroid women with thyroid autoimmunity (TAI).

Materials and Methods A comparative analysis of ART outcomes of 119 women with TAI and 128 women without thyroid pathology.

Results From 1–6 IVF failures were registered in 30 cases (25%) of TAI and 36 (28.1%) patients without thyroid (T) pathology (p > 0.05). Nevertheless, IVF attempts distribution in both groups demonstrated the fact that 1–2 IVF attempts in the anamnesis were found in 46.7% of women with TAI and 77.8% of the controls (p > 0.05), meanwhile more than six repeated IVF failures (3 and more) was noted by more than a half of the studied cohort of women (53.3%) and only by every 5th patient of the control group (22.2%; p < 0.05).

Assessment of follicular genesis, oogenesis, and early embryogenesis in IVF programmes of both groups demonstrated a reliable decrease of an average number of aspirated follicles (5.9 ± 1.2, 7.6 ± 1.5; p = 0.0412), average number of blastocysts (1.6 ± 0.2 and 2.4 ± 0.3; p = 0.0246), and percentage of blastocyst formation (70.8% and 92.3%; p = 0.0282) in patients with TAI comparing with those without T pathology.

Pregnancy rate calculating to embryo transfers made up 22.2% in patients with TAI that was 1.5 times as less comparing with the cohort without T pathology (32.4%; p < 0.05). Reproductive losses were reliably higher in patients with TAI as compared with women without T pathology (19.2% and 6.5% correspondingly; p < 0.05).

Conclusion Unfavourable ART outcomes, reliable low indexes of follicular genesis and embryogenesis, low IVF programmes efficacy and a high rate of reproductive losses are typical for women with infertility and thyroid autoimmunity comparing with patients without thyroid pathology.

FC 4-3 Reproductive endocrinology

Circulating level of stem cell factor in serum of hyperstimulated IVF patients

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Introduction Stem cell factor (SCF) play a major role in hematopoiesis and spermatogenesis, and possibly female fertility.

Materials and Methods In group 1 (n = 75) we analyzed SCF levels in serum and follicular fluid (FF) on the day of follicular puncture (FP). In response to ovarian stimulation, SCF levels in serum and in FF were compared between low (n = 25), moderate (n = 26) and high (n = 14) response patients. In group 2, 30 patients serum for SCF assessment was collected throughout menstrual cycle until gestation.

Results The level of SCF related to the number of follicle (f) in serum and in FF decreased from low through moderate to high response (p < 0.001); pregnancy rates were 20, 34.6 and 50.1% respectively (p = 0.001). SCF in serum increased gradually from stimulation day (st) 6–8 through st. 9–11 and reached a peak on the day of hCG injection (p < 0.04). On the day of FP the SCF levels dropped slightly. On post retrieval days, SCF in serum from FP through ET, implantation to the day of confirmation of pregnancy, the SCF levels increased significantly and reached their highest level (p = 0.02). After implantation the SCF level decreased slightly and reached a plateau during gestation. Interestingly SCF levels in ICSI-treated patients (n = 20) in the luteal phase (ET+4w) were significantly higher than in patients treated with IVF (n = 10; p < 0.05).

Conclusion SCF is involved in follicle development and may be a predictor of IVF outcome.

FC 4-4 Reproductive endocrinology

Peritoneal fluid leptin level in women with unexplained infertility

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Introduction Leptin is an adipocyte hormone, and a type-1 cytokine with a possible role in reproductive function. Unexplained infertility is a diagnosis of exclusion, in the face of normal workup of an infertile couple. Peritoneal fluid (PF) is a biologically active environment with changes in PF composition that can influence reproductive function.

Objective To estimate the levels of leptin in peritoneal fluid of women with unexplained infertility and compare them with those from fertile women.

Study Design Prospective case control study.

Materials and Methods Peritoneal fluid was obtained from 51 women undergoing laparoscopy for unexplained infertility (cases) or ligation (controls). Infertile women were recruited if the couple had normal results on routine workup. Women with active pelvic inflammatory disease, endometriosis, PCOS, obesity (BMI > 30 kg/m2) were excluded from the study. All samples were obtained during the follicular phase of the menstrual cycle and analyzed using commercially available ELISA kit.

Results PF samples were obtained from 23 women with unexplained infertility and 28 fertile women undergoing tubal ligation. The mean age of women with unexplained infertility was 30.3 (± 3.2) years and 28.5 (± 6.2) years in the fertile women. The BMI of the unexplained infertile women and fertile controls was 25.1 (± 4.2) Kg/m2 and 23.3 (± 4.2) Kg/m2 respectively. There was no significant difference in the age and BMI among women with infertility and fertile controls. The mean PF leptin levels were significantly higher in women with unexplained infertility (5.3 ng/ml (1.4–20)) in comparison to fertile women 2.0 ng/ml (0.1–11) (p < 0.001). On using simple regression analysis to test the correlation of PF leptin levels with BMI between infertile and fertile women, there was significant correlation of PF leptin levels with BMI (p = 0.015). Thus for a given BMI PF leptin levels were significantly higher in women with unexplained infertility than PF levels in fertile women.

Conclusions A significant difference in the PF leptin levels between unexplained infertile and fertile women suggest that this
cytokine may be involved in the patho-physiology of unexplained infertility.

**FC 4-5 ART clinical**

Low-dose metformin improves pregnancy rate in IVF repeaters without polycystic ovary syndrome, who are selected by multiple insulin-resistance-related parameters

**Objective** Insulin resistance is associated with aging and stress. These two factors are common among patients who repeatedly fail to conceive with in vitro fertilization (IVF) repeaters. First, we examined whether and how administration of low-dose metformin improved IVF outcome in IVF repeaters without polycystic ovary syndrome (PCOS), and defined indications for this therapy. Next, we performed a prospective randomized study to confirm the effectiveness of low-dose metformin therapy in IVF repeaters without PCOS, fulfilling its indications.

**Materials and Methods** We studied 233 women without PCOS who had failed at least twice to conceive in previous IVF. Metformin (500 mg/day) was administered for 8 to 12 weeks before and during ovarian stimulation for IVF (metformin IVF). In study I, IVF outcomes with metformin (n = 33) were compared to outcomes without metformin in the patients’ previous IVF as well as to outcomes in control patients matched to metformin patients by age, body mass index, and previous IVF failures. A discriminant score (DS) was developed from nine insulin-resistance-related parameters obtained before metformin administration to predict achievement of ongoing pregnancy by metformin IVF. In study II (n = 199), the rates of ongoing pregnancy was prospectively compared between four groups divided at random by DS above/below 0.6647 and with/without metformin.

**Results**

Study I: Follicular development was significantly better with metformin than previously, and significantly more high-quality embryos were obtained with metformin. The rate of ongoing pregnancy was significantly higher with metformin (30% in 33 cycles) than in previous IVF (1.9% in 104 cycles) or matched controls (6.1% in 33 cycles). Ongoing pregnancy by metformin IVF was predicted with 0.90 sensitivity and 0.91 specificity by DS, using optimal threshold of 0.6647.

Study II: The rate of ongoing pregnancy was significantly higher in women with DS above 0.6647 with metformin (56% in 18 women) than in ones with DS below 0.6647 without metformin (20% and 15% in 15 and 86 women, respectively).

**Conclusions** Low-dose metformin improves pregnancy rate in IVF repeaters without PCOS, most likely by decreasing insulin resistance. Its indication can be decided by DS calculated from nine insulin-resistance-related parameters before metformin administration.

**FC 4-6 Reproductive endocrinology**

The orphan nuclear receptor NR4A1 regulates transcription of key steroidogenic enzymes in ovarian theca cells

**Aims** Orphan nuclear receptor NR4A1, a member of the nuclear receptor superfamily, is widely expressed in different cell types and mediates diverse physiological processes. Recent emerging evidence suggests that NR4A1 is involved in the transcriptional regulation of several steroidogenic enzyme genes in gonads and adrenals. However, its function in ovarian theca cells remains to be defined.

**Methods** In the present study, immunohistochemical staining of NR4A1 was conducted to investigate the localization in healthy human ovaries. In an effort to explore the function of NR4A1 in the transcript regulation of steroidogenic enzyme genes responsible for ovarian theca cell steroidogenesis, we constructed recombinant adenovirus AdCMV-NR4A1 and AdH1-NR4A1 to enhance or knockdown the expression of NR4A1 in theca cells, respectively. The expression patterns of StAR, CYP11A1, CYP17 and HSD3B2 were subsequently analyzed by real-time RT-PCR. Moreover, concentrations of testosterone in the spent medium were measured by radioimmunoassay. Moreover, since expression of NR4A1 in the endocrine organs is known to be regulated by both cAMP/PKA mediated hormones, ACTH and LH, forskolin (FSK), an activator of cAMP/PKA pathway, was applied to the cultured follicles.

**Results** NR4A1 was expressed in theca cells and granulosa cells in healthy human ovaries by immunohistochemistry. Overexpression of NR4A1 in theca cells stimulates the expression of StAR, CYP11A1, CYP17 and HSD3B2, leading to increased testosterone production. Conversely, knockdown of the endogenous NR4A1 exhibits a significant decrease in StAR, CYP11A1, CYP17 and HSD3B2 expression and testosterone production. FSK rapidly increases the NR4A1 mRNA levels followed by an increase in StAR, CYP11A1, CYP17 and HSD3B2.

**Conclusion** Our results outline a previously unrecognized role for NR4A1 in the transcriptional regulation of StAR, CYP11A1, CYP17 and HSD3B2 in ovarian theca cells. Modulation of these steroidogenic enzymes by NR4A1 could influence the capacity of the ovarian theca cells to produce androgen.

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**FC 4-7 Reproductive endocrinology**

Prediction of pregnancy in PCOS women treated with metformin – results of a multicentre placebo-controlled randomised trial

**Introduction** The role of metformin in the treatment of infertility in women with polycystic ovary syndrome (PCOS) is still controversial. Small RCTs have shown a significant improvement of pregnancy rates (PRs), but two large RCTs failed to show any beneficial effect on PRs or live birth rates (LBRs). Our recent RCT, however, showed a significant improvement of PRs and LBRs with metformin compared to placebo.

The aim of this study was to investigate whether the clinical outcome during/after metformin treatment in PCOS can be predicted based on the baseline hormonal and metabolic parameters of the patients at the time of metformin treatment, i.e., to find the women who may benefit from metformin therapy.

**Materials and Methods** 315 women with PCOS and anovulatory infertility were randomised to metformin (Diformin®, Leiras, Finland) or placebo. Obese women (body mass index [BMI] > 27 kg/m²) received 1000 mg 2 x daily and non-obese subjects (BMI < 27 kg/m²) 500 mg 3 x daily, or an identical dose of placebo. After 3 months of treatment with metformin/placebo alone, another appropriate infertility treatment (clomiphene, gonadotrophins, insemination, IIVF/ICSI) was combined, if necessary. If pregnancy occurred, metformin/placebo was continued up to the 12th week. Blood samples were drawn and oral glucose tolerance test (75 g) was performed at 0 and 3 months of treatment, and at the 7–8th week of pregnancy.

**Results** Metformin and placebo groups did not differ at baseline as regards clinical, hormonal or metabolic parameters. At 3 months of treatment, BMIs (26.4 ± 6.0 vs 28.9 ± 11.1 kg/m²; p = 0.04), serum levels of test-
Spermatogenesis is the process of sperm cell development. This process involves precise control of germ cell proliferation and death by several factors including hormones, and mediators synthesized by Sertoli cells (SC). Classically viewed as female hormones, estrogens have been shown to play important roles in the control of male reproduction. In fact 17β-estradiol (E2) has been identified as a germ cell survival factor, although the precise mechanism by which E2 exerts this effect remains unknown so far. Recently, using suppressive subtractive hybridization, our research group has identified several novel estrogen-regulated genes in rat testis, two of which might play a role in the regulation of apoptotic cell death. One is regucalcin (RGN), also called senescence marker protein 30, a calcium binding protein which regulates intracellular calcium concentration by enhancing the calcium pumping activity of the plasma membrane and has been shown to inhibit apoptotic cell death in several cell types. The other is Aven, a caspase inhibitor that acts by enhancing anti-apoptotic Bcl-xL and inhibiting pro-apoptotic Apaf-1 self-assembly. Recently it has also been shown that Aven acts as a transducer of DNA damage signaling by regulating cell-cycle arrest. The aim of the present work was to confirm RGN and Aven expression-regulated genes. In this study we investigated seminiferous tubules cultured ex vivo, and to determine the localization of RGN and Aven proteins in human and rat testis by immunohistochemistry (IHC). Using quantitative reverse transcriptase-polymerase chain reaction we have demonstrated that both RGN and Aven are regulated by E2 in rat seminiferous tubules. By IHC we have localized RGN protein to SC and Leydig cells, spermatogonia, spermatocytes, spermatids and sperm tails. Aven was localized to SC and to germ cells up to the round spermatid level, with strong staining of spermatocytes. Also, we were able to confirm the presence of RGN and Aven in rat and human cultured SC. The results presented here provide evidences of a possible link between estrogen and apoptosis in tests and highlight the importance of estrogens in male reproductive physiology.

### FC 5: PGD & reproductive genetics

#### FC 5-1 Pre-implantation genetic diagnosis

**Chromosome length correlates with aneuploidy**

Schmutzler R.G., Schmitz N., Borghese J.E.*

**Introduction** Implantation and intrauterine growth biases to the analysis of aneuploidy are overcome by blastomere biopsy of embryos in vitro for PGS - but not the bias of fertilization. This can only be overcome by diagnosis of oocytes. Results from first and second polar bodies must be analyzed separately in order to understand possible reasons for intracellular malfunctions, e.g. of the spindles. Finally biases of origin for those malfunctions, i.e. especially age, should be excluded.

**Materials and Methods** After approval of the local ethics committee, we performed 104 PGS cycles between 2004 and 2008. PGS was offered to all patients with 8 or more oocytes, independent of age or other indications. It was performed by laser biopsy of the first polar body on day 0, fixation for FISH and hybridisation with chromosomes 13, 16, 18, 21, 22 (Vysis). For each chromosome the number of chromatids present in the first polar body was noted: 0 (lack of 1 chromosome), 1 (lack of one chromatid), 2 (normal), 3 (surplus of one chromatid), 4 (surplus of one chromosome).

**Results** The average age of the women was 34 years (range 23–43 years). 1285 oocytes were retrieved, i.e. in average 12 per patient. Of these 1150 were mature (89%), of these 893 (78%) were biopsied, of these 833 (93%) survived. Of the 893 biopsied oocytes 778 (87%) polar bodies could be fixed, of these we obtained genetic results for 621 (80%) oocytes: 227 (37%) were euploid, 394 (63%) aneuploid. On the 621 aneuploid oocytes investigated we got 2495 single results. (1) Aneuploidy rates for chromosomes 13, 16, 18, 21, 22 were 26%, 17%, 16%, 30%, 31%, respectively. These differences were statistically significant (p < 0.01). (2) Malsegregation of chromosomes Aneuploidy Screening (56, 68, 31, 37 % of the aneuploidies, respectively (p < 0.01).

**Conclusions** 1. Chromosomes 21 (50 MB) and 22 (56 MB) show an aneuploidy rate (30–31%) about double the size than chromosomes 16 (98 MB) and 18 (85 MB) (16–17%). Aneuploidy correlates negatively and proportionally with the length of the chromosome.

2. Single chromatin multialteration correlates positively and proportionally with the length of the chromosome.

3. Chromosome 13 (114 MB) for both seems to be an exception.

4. In sum spindles seem to have a tendency to missort chromosomes in first meiosis the smaller they are and chromatids the bigger they are!

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**FC 5-2 Reproductive genetics**

The role of morphological nuclear integrity of the sperm cells in pre-implantation genetic aneuploidy screening cycles outcome

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**Introduction** The aim of this study was to evaluate the effect of sperm morphology on embryo development at chromosomal level.

**Materials and Methods** Couples who underwent their first in vitro fertilization (IVF) cycle in conjunction with Preimplantation Genetic Aneuploidy Screening (PGS), as result of advanced maternal age, were randomly allocated in two groups: ICSI (n = 60), in which the sperm cell was examined at high magnification from ICSI cycles when compared to 400X; and IMSI (n = 60), in which all biopsied embryos were analyzed for chromosomes X, Y, 13, 16, 18, 21 and 22.

**Results** Our results indicated that there is a significantly increased incidence for sex chromosomes aneuploidy on embryos arising from ICSI cycles when compared to IMSI cycles (23.5% vs 15.0%; p = 0.0139). Regression analysis showed that high magnification sperm selection was associated with a significantly lower risk of sex chromosome abnormalities (OR: 0.57, CI: 0.37–0.90; p = 0.015). The incidence of chaotic embryos was also significantly higher on ICSI cycles (27.5% vs 18.8%; p = 0.0193) and IMSI procedures was associated with a significantly lower risk of chaotic embryos (OR: 0.64, CI: 0.43–0.96; p = 0.032). Although pregnancy...
Premature ovarian failure (POF) is generally defined as secondary amenorrhea characterized by hypergonadotropic hypogonadism affecting 1–2% of the general female population prior to age of 40 years. Genetic diseases affecting 1–2% of the general female population are defined as secondary amenorrhoea characterized as hypergonadotropic hypogonadism.
cations. Embryo transfer was performed in 35 out of 48 cycles (72.9%), 83.3% (20/24) in Robertsonian and 62.5% (15/24) in reciprocal translocations. 12 pregnancies were achieved (25% per cycle, 34.3% per transfer), 29.1% per cycle and 35.0% per ET for Robertsonian and 20.6% per cycle and 33.3% per ET for reciprocal translocation. Only one pregnancy in each group ended as abortion.

Discussion
Comparative pregnancy rates per embryo transfer in couples with Robertsonian and reciprocal translocation could be achieved. However, in couples with reciprocal translocation significant less "normal" embryos were found and significant higher percentage of cycles was cancelled because of all embryos being abnormal.

Summary
In couples with translocation and infertility problems IVF-PGD allows in short term a conception and delivery of a healthy baby. Couples with Robertsonian translocation have in general better prognosis compared to couples with reciprocal translocation.

Introduction
In translocation carriers oocyte segregation during meiosis I forms so called quadrivalents (in reciprocal translocation) or trivalents (in Robertsonian translocation) of the translocated chromosomes besides the regular homologue chromosomal pairing. Theoretically there could arise 16 different or 6 different chromosomal constitutions in secondary oocytes whereas only two of them are balanced. Those balanced oocytes carry an additional risk for aneuploidies, possibly even higher than age-related risk.

Aim
The aim of this evaluation is to determine the proportions of the different segregation patterns in oocytes of translocation carriers as well as the rate of aneuploidies respectively euploid and balanced oocytes.

Methods
We performed polar body analysis as an indirect method to evaluate meiosis I in oocytes by using FISH on first polar bodies. Probes were selected according to the chromosomes involved in the translocation and up to 5 additional chromosomes. Here we present our result from 27 cases (25 reciprocal and 2 Robertsonian translocations).

Results
Out of 180 clearly evaluable polar bodies, 77 were balanced and 103 were unbalanced. The following segregation patterns were observed: 12 adjacent 1-, 7 adjacent 2- and 24 3:1-distributions. A 4:0 segregation was not revealed. The results of 60 oocytes could not be assigned to the segregation patterns above, but a trisomy or monosomy of one or both of the translocated chromosomes was predicted. This applies to all Robertsonian translocations or was due to lack of availability of appropriate FISH probes or inconclusive signals. In respect to the aneuploidy diagnostic we revealed the following ratio for the balanced polar bodies: 31.4% aneuploid and 68.6% euploid, and for unbalanced polar bodies: 65.4% aneuploid and 34.6% euploid.

Conclusions
Our results show a surprisingly high rate of balanced segregation. The percentage of adjacent 1 or adjacent 2 distribution seems low, while the 3:1 segregation is the most prevalent. This is dependent from the localisation of the break points, which predicts the asymmetry of the quadrivalent. We could also show that the risk for an additional aneuploidy is much higher for oocytes with an unbalanced than with a balanced segregation for the translocated chromosomes.

FC 5-8 Reproductive genetics
Protemeic analysis of follicular fluids from patients with the 5,10-methylenetetrahydrofolate reductase 677C>T mutation
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Background
A glutamatic acid decarboxylase (GAD65) autoantibody is present in patients with type 1 diabetes (T1DM) and in autoimmune polyendocrinopathies. In the present study we investigated, whether the GAD65 autoantibody is a marker for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APC). Patients with T1DM and healthy controls were recruited from the Diabetes Center at the Ludwig-Maximilians-University Munich.

Methods
After signing the informed consent, patients with T1DM were included in the study. Antigen-specific (anti-GAD65) and idiotype-specific (anti-idiotype) antibodies were analyzed by enzyme-linked immunosorbent assay (ELISA) using recombinant human GAD65, which is an extracellular enzyme secreted in the urine of diabetic patients and healthy controls.

Results
In the present study, we investigated 63 patients with T1DM and 23 healthy controls. Anti-GAD65 antibodies were detected in 22 of 63 patients (34.9%) with T1DM and in 1 of 23 healthy controls (4.3%). Anti-idiotype antibodies were detected in 1 of 63 patients with T1DM and in 0 of 23 healthy controls. The prevalence of anti-GAD65 antibodies in patients with T1DM was significantly higher compared to healthy controls (p < 0.05).

Discussion
The present study suggests that anti-GAD65 antibodies may be a marker for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APC) in patients with type 1 diabetes (T1DM). Further research is needed to confirm these findings and to determine the clinical relevance of anti-GAD65 antibodies in patients with T1DM.
FC 6: Infertility surgery

FC 6-1 Infertility surgery
Premature ovarian failure and diminished ovarian reserve following surgical treatment of polycystic ovarian disease (PCOD)

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Objective To highlight the possibility of premature ovarian failure and diminished ovarian reserve following surgical therapy of polycystic ovarian disease.

Design Case series.

Materials and Methods 14 women aged between 21–39 years presented to us with markedly diminished ovarian reserve following surgical treatment for polycystic ovarian disease. 13 of them had bilateral laparoscopic ovarian drilling and one patient had bilateral wedge resection of the ovaries through minilaparotomy.

Results 7 patients had premature ovarian failure (secondary amenorrhea and FSH > 40 miu/ml) diagnosed 3 to 18 months following the procedure. The remaining 7 patients had markedly diminished ovarian reserve during the same period of time (Oligo-amenorrhea, FSH levels between 12 and 30 miu/ml).

Conclusions Premature ovarian failure and diminished ovarian reserve are the serious complications of surgical treatment of PCOD. Such cases and complications are mostly unpublished. Accurate and documented diagnosis of PCOD, appropriate surgical training and adjusted number of punctures are essential for the avoidance of such catastrophic complications.

FC 6-2 Infertility surgery
Robotic assisted laparoscopic myomectomy using the Da Vinci system

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Study Objective To report patients who underwent Robotic Assisted Laparoscopic Myomectomy using the Da Vinci robotic system and the factors affecting the total operative time, console time and total blood loss.

Setting Tertiary care facility.

Intervention Robotic Assisted Laparoscopic Myomectomy was administered.

Subjects and Method Between February 2007 and January 2009, 70 consecutive patients underwent robotic laparoscopic myomectomy and complete data was obtained from 51 patients. Data included total operative and console time, estimated blood loss, length of hospital stay, number of leiomyomata, diameter of the largest tumor, age and BMI.

Results The operative time range was 98–408 min and the mean was 204.05 ± 67.93 min (95%-CI: 186.41–222.69 min). The Console time range was 27–200 min and the mean was 99.45 ± 46.29 min (95%-CI: 86.76–112.15 min). The blood loss range was 50–300 ml and the mean was 104.60 ± 56.77 ml (95%-CI: 89.02–120.18 ml). The number of leiomyomata range was 1–6 and the mean was 2.88 ± 1.27 (95%-CI: 2.53–3.23). The tumor size range was 17.5–88 mm and the mean was 43.16 ± 17.58 mm (95%-CI: 38.39–47.93 mm). The length of the hospital stay range was 0.4–2 days and the mean was 1.04 ± 0.25 days (95%-CI: 0.97–1.11 days). The age at the time of surgery range was 23–57 years and the mean was 38.84 ± 6.58 years (95%-CI: 37.03–40.29 years). The BMI range was 14.2–35.7 kg/m² and the mean was 26.51 ± 4.47 kg/m² (95%-CI: 25.28–27.74 kg/m²).

Tumor size had a significant positive impact on surgery time (p = 0.0205) and that number of tumors had a positive significant impact on console time (p = 0.0246). We also found that tumor size, number of leiomyomata, console time, and non console time, had significant increase on blood loss with p-value of 0.0031, 0.0181, 0.0001, and 0.0002 respectively.

Conclusions Da Vinci robotic equipment has made the laparoscopic Myomectomy procedure more precise, and allows the surgeon’s hand movements to be scaled, filtered and translated into precise movements of micro-instruments within the operative field. In this study, a positive significant impact of the tumor size on total operative time, a positive significant impact of the number of leiomyomata on console time and a positive significant impact of tumor size, number of leiomyomata, console time and non console time on the total blood loss, were demonstrated.

FC 6-3 Infertility surgery
IVF outcomes after office hysteroscopy

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Objective To evaluate the incidence of intrauterine pathology in patients undergoing IVF treatment. In addition, to evaluate the effectiveness of office hysteroscopy in the treatment of intrauterine pathology identified.

Methods Prospective study 180 patients undergoing IVF treatment. The group study included 120 patients with one or more failed IVF cycles and 60 patients without IVF attempts.

Results Various pathological conditions were found in 75 of cases (41.6%), among which an endometrial polyps 26 (34.6%), submucous myoma 12 (16%), intrauterine synchie 4 (5.3%), chronic endometritis 13 (17.3%), endometrial hyperplasia 18 (24%). In 35% of cases this pathology of the endometrium is not diagnosed before the hysteroscopy any other diagnostic methods, which confirms the diagnostic value of office hysteroscopy. Patients were divided into 2 groups according to hysteroscopy findings. The patients who had no pathology were included in Group 1 (n = 105) and patients who had abnormal hysteroscopic findings were included in Group 2 (n = 75). In 36 (48%) pathological findings removed during office procedure, in 39 (52%) required D&C, hysteroscopic, hormonal therapy. Pregnancy rate are comparable among the normal and surgically correction groups. There was no significant difference in the clinical pregnancy rate between patients in groups 1 and 2 (19.4% and 24.1%).

Conclusions Diagnostic and medical outpatient hysteroscopy has the following advantages: lower cost compared to hospital hysteroscopy, no requirement for general anaesthesia (anesthesiologist, nurses, lower risk for the patient). Hysteroscope’s small external diameter allows to conduct most diagnostic and therapeutic procedures without stretching of the cervical canal. In general, the procedure is well tolerated, in rare occasions satisfactorily. The procedure takes from several minutes to half an hour. It also provides a “presence” feeling to the patient as she can watch the procedure on the monitors. Office Hysteroscopy, as a routine examination, should be performed before the IVF cycle in all patients. The incidence of pathologic findings on hysteroscopy is high in patients undergoing IVF. Office Hysteroscopy is also the best way to repair the uterine cavity when pathological conditions are present.

FC 6-4 Infertility surgery
Can open tubal microsurgery be still helpful in tubal infertility treatment?

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During 30 years (1977–2007), 1669 patients underwent open microsurgery for tubal diseases.

Adhesiolysis, reanastomosis, fimbrioplasty, salpingonecostomy, proximal reconstruction: isthmo-ostial anastomosis and reimplantation are described.

Results were excellent for patients with a favourable prognosis (1517 patients): very high pregnancy rate: 80% pregnancies with vaginal delivery for tubal reversal, 68% for proximal diseases, 75.1% for fimbrioplasty, 55% for...
salpingo-oophorectomy; very low risk of ectopic pregnancy: 1.5% for reversal, 4% for proximal surgery, 4% for fimbrioplasty and 6.7% for tubal ligation. Results were very bad for patients with a poor prognosis (152 patients), after 1987 we abandoned reconstructive surgery for these patients and recommended salpingectomy to increase favourable results for IVF.

Open microsurgery can still be helpful in tubal infertility treatment because results are still better than laparoscopic reconstructive surgery results and better than IVF results in case of patients with a favourable prognosis. Patient is operated only one time and can have several pregnancies.

**FC 6-5 Infertility surgery**

**Endometriosis: A new approach**

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**Background** Endometriosis is a common health problem in women. 14% of all women suffer endometriosis and 30–50% of infertile women have endometriosis. To evaluation of new approach for treatment of endometriosis this study was carried out.

**Methods** In one year period, all women referred to our department were evaluated for endometriosis. 47 infertile women were diagnosed as endometriosis cases and were underwent first laparoscopy that endometriosis spots destroyed by diathermy. They were treated with monthly injection of Triptorelin Acetate 3.75 mg after laparoscopy for three months. After third month, endometriosis was evaluated by second laparoscopy (Second Look) and if endometriosis existed, medical treatment was continued for another three months. After six month medical therapy, the third laparoscopic evaluation (Third Look) was conducted. If endometriosis still existed, after three month more treatment, the laparoscopy or laparotomy was done.

**Results** 10 (21.3%) cases had endometriosis grade I (according to classification of the American Society of Reproductive Medicine, ASRM), 20 (42.6%) cases grade II, 10 (21.3) cases grade III and 7 (14.8%) cases grade IV. 9 of 10 (90%) of grade I patients were cured after three months treatment (Second Look). Another patient in grade I (10%) was cured after six month medical therapy (Third Look). Patients with grade II (16 cases) and grade III (5 cases) were cured after three month medical therapy (80% and 40% respectively). All remaining patients in grade II and III were cured after six month treatment. 3 of 5 cases of grade IV endometriosis were underwent surgery (laparoscopy) for resolving adhesions after 3 month medical therapy. 2 cases were underwent laparotomy for remodelling and constructing of uterus and pelvic organs and referred for assisted reproductive techniques (ART).

**Conclusions** This study shows that many patients with low to moderate endometriosis (grade I, II and III) may be cured after first laparoscopic diathermy of lesions and subsequent medical treatment for three or six months. Patients with high grade (grade IV) may respond difficultly to medical therapy and may need to widespread laparoscopic or laparotomy surgeries for treatment or improvement of function of pelvic organs. It may needs to performing assisted reproductive techniques (ART) for treatment of infertility in patients with high grade endometriosis.

**FC 6-6 Infertility surgery**

**The first successful live birth following a uterine allo-transplant**

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**Objective** To demonstrate that a pregnancy can be achieved in a uterine allograft in the sheep model with the guidance of assisted reproductive technology.

**Methods** We conducted a pilot study in a series of 12 sexually matured African sheep. The study took place at the Universidad de La Salle Bogota, Colombia from March 2008 to February of 2010. A control group of pregnant Romney Marsh sheep with a non-transplanted uterus were used to compare fetal development, uterine/placental histology and blood samples of progeny with the uterine transplanted sheep. Fetal sizes were obtained from ultrasonographic measurements during the early (crown-rump length) and late (biparietal diameter, abdominal circumference) gestational periods. The primary endpoint variables included pre and post-operative management, embryo transfer protocol, intra-operative assessments and the physiologic cardio-pulmonary changes in the lamb during the first 5 hours of life.

**Results** 4 months after the initial uterine transplant, 5 out of the twelve uterine allografts were considered candidates for the embryo transfer procedure. Fresh and frozen blastocysts donors were transferred according to the remaining 5 uterine allografts through a mini-laparotomy incision. 3 of these 5 resulted in pregnancies where one developed an ectopic gestation while the second carried a pregnancy to 105 days. The third delivered a fully developed offspring from the transplanted uterus by cesarean section. Neonatal lamb blood-gases, tissue histology, ventilation and pressure flow studies documented normal for gestational age.

**Conclusion** In this first reported case in the medical literature, we have demonstrated that a pregnancy can be carried in an allografted uterus with the end result of a successful delivery.

Surgical instruments were supported by Apple Medical, and the Research was funded by a family cooperation (RAFAM, Inc.).

**FC 6-7 Infertility surgery**

**Surgical intervention to overcome difficult cervical canal negotiation encountered during embryo transfer**

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**Introduction** The use of invasive interventions to overcome a difficult embryo transfer (ET) has been shown to improve outcome of in vitro fertilization (IVF). Cervical dilatation done prior to the cycle is not helpful in all patients.

**Objective** To evaluate whether surgical correction of cervical canal abnormalities with versapoint could improve ease of embryo transfer in patients who did not respond to cervical dilatation.

**Materials and Methods** Study included 11 patients with history of very difficult ET despite going through cervical dilatation prior to their IVF cycle. During previous ET negotiation of the cervical canal had to be carried out ultimately with a metal outer sheath. Blood was present on the transfer and outer catheter in all patients. Hysteroscopy had revealed the following cervical canal abnormalities, false passage in the cervix in 3 patients, tortuous cervical canal in 5 and a severely fibrotic os in 3.

**Procedure** Patients were taken up for hysteroscopy using a versascope. Versascope was guided into the cervical canal without prior dilatation. In patients with fibrotic os 1 or 2 linear releasing incisions were made with the versapoint electrode, extending from the posterior aspect of the internal os towards the external os for 1 cm. In patients with a tortuous cervical canal several projecting ridges were seen arising from the anterior, posterior and/or lateral walls of the cervical canal, linear incisions were made into these projections and a straightening of the canal was achieved. When a false passage was identified tissue between the actual canal and false passage was cut thus leaving a clean path. After the procedure a mock embryo transfer was performed to confirm adequacy of procedure. There was no bleeding or complications. A mock ET was performed 4 weeks after the surgery.

**Results** No difficulty was encountered in mock ET. Subsequent IVF-ET done in all the 11 patients was smooth and there was no blood on the catheter. 5 of the 11 patients achieved pregnancy.

**Conclusions** Surgical correction of the cervical canal in patients having difficult em-
FC 7: Ovarian stimulation

FC 7-1 Ovarian stimulation

Letrozole with gonadotrophins improves success rates in PCOS patients undergoing intrauterine insemination

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Objective To evaluate the use of letrozole in patients undergoing superovulation and IUI in PCOS group of patients and compare their efficacy with gonadotrophin superovulation.

Materials and Methods Case controlled cohort study in 2 groups of PCOS patients letrozole and FSH and gonadotropins alone and undergoing intra-uterine insemination.

Main Outcome Measures Fertility Research Center.

Patients PCOS women less than 40 years and infertility more than 1 year undergoing IUI.

Interventions The first group received letrozole 5 mg from day 3 to day 7 and gonadotropins from 75 IU from day 5 until the dominant follicle reached 18 mm and ovulation was triggered with 5000 IU hCG. The second group received gonadotropins alone from day 3 of cycle till the dominant follicle was 18 mm and ovulation were triggered with hCG 5000 IU. Intrauterine insemination was performed 36 hours and 48 hours after hCG administration.

Results Intrauterine insemination was performed in 111 cycles in PCOS patients were conducted in a period of 12 months from Jan 2009–December 2009. Letrozole plus gonadotropins was used in 61 PCOS patients undergoing IUI, and gonadotropins alone was used in 50 patients with PCOS undergoing IUI. 25 out of the 61 patients undergoing IUI with letrozole and gonadotropins conceived (40.98%) and 11 out of the 50 patients receiving gonadotropins conceived (22%). Patents cotreated with letrozole required fewer gonadotropins, developed more follicles larger than 14 mm. PCOS women with BMI less than 30 had significant pregnancy rates compared with PCOS having BMI more than 30. The endometrial response was significantly better in letrozole plus gonado- trophin group; p-value 0.03 out of the 2 groups. The pregnancy rate in PCOS undergoing intrauterine insemination with letrozole plus gonadotrophin group was 40.98% compared to letrozole group (22%); p-value 0.1.

Conclusions The addition of letrozole to gonadotrophins in PCOS patients undergoing IUI decreases gonadotrophin requirement, increases preovulatory follicles, with no negative effect on endometrium.

FC 7-2 Ovarian deficiency

Clinical and karyotype analysis of Chinese women with premature ovarian failure

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Aims Premature ovarian failure (POF) affects about 1% of women before 40 years old and causes infertility and primary or secondary amenorrhea. The phenotypes of POF patients are variable and etiology of POF is mostly unknown. The natural history of the clinical manifestations of POF remains poorly understood, and there have been few previous large-scale studies of this condition.

Methods We performed a mixed prospective and retrospective study of clinical, biochemical and genetic data relating to 283 Chinese Han women with POF between 2007 and 2009.

Results The mean age at the time of diagnosis was 29.4 ± 4.7 years. 83% (235/283) presented with secondary amenorrhea, of which 66 subjects displayed secondary infertility (28.1%, 66/235). 26 (9.2%) patients had a family history of POF or early menopause. 6 patients (2.1%) presented with a clinical autoimmune disease (Psoriasis, Hashemite thyroiditis, Behcet disease and thyroid hypofunction). Biochemical autoimmune factors (anti-thyroglobulin antibody, anti-thyroid peroxidase antibody, anti-adrenocortical antibody, anti-cardiolipin antibody, antinuclear antibody and dsDNA antibody) were found in 17 out of 48 patients (35.4%). The incidence of anti-adrenocortical antibody positive was significantly higher than controls, 5% (15/283) suffered from pelvic surgery before the diagnosis of POF. The incidence of epidemic parotitis in POF (13.4%) was significantly higher than that in domestic general population. Karyotype anomalies were observed in 16.9% (28/166) and 22 of which had X chromosome abnormalities. 6 other patients had autosomal abnormalities.

Conclusions Genetics, infection, autoimmunity and pelvic surgery contribute to the etiology of POF to a certain extent in Chinese population. It is important to preserve the ovarian function during pelvic surgery and put emphasis on vaccination and treatment of epidemic parotitis. POF patients mostly presents as secondary amenorrhea with a disturbance in a previously established menstrual cycle. It could be inferred that part of POF patients are due to accelerated exhaustion of primordial follicles. To get pregnant as early as possible should be recommended for women with high risk of POF.
This study was supported by National Natural Science Foundation of China (30973170).

**FC 7-3 Ovarian stimulation**

*Poor response following ovarian stimulation affects embryo multiple abnormalities occurrence in ICSI cycles*

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**Introduction**

Ovarian poor response to stimulation protocols increases as women get older and embryos from these poor prognosis in-vitro fertilization (IVF) patients were proposed to have higher aneuploidy rates. The aim in this study was to evaluate if aged women with poor ovarian response express an increase on embryo chromosomal alterations.

**Methods**

Couples undergoing intracytoplasmic sperm injection (ICSI) cycles associated to preimplantation genetic screening (PGS), as a result of advanced maternal age, were subdivided into 2 groups: Poor Responder (PR group, n = 34), patients who produced four or less oocytes; and Normo-responder (NR group, n = 50), patients in which 5 or more oocytes were retrieved. After ICSI, all the biopsied embryos were analyzed for chromosomes X, Y, 13, 16, 18, and 21. Groups were compared regarding ICSI outcomes and aneuploidy frequency.

**Results**

There were no significantly differences on fertilization (73.9% vs 76.6%; p = 0.7861), implantation (22.6% vs 20.6%; p = 0.6863) and pregnancy rates (28.0% vs 26.4%; p = 0.0920) between NR and PR groups; however, a significantly increase on the mean number of transferred embryos (2.0 ± 1.0 vs 1.1 ± 0.8; p = 0.0047) was observed on NR group. Regression analysis showed no significant influence of poor response on the percentage of embryos showing autosomal aneuploidy (OD: 1.23, CI: 0.74–2.05; p = 0.417), sexual aneuploidy (OD: 0.9, CI: 0.44–1.85; p = 0.769). On the other hand, poor response was associated with a significantly increased risk of embryo multiple abnormalities occurrence (OD: 2.15, CI: 1.15–4.04; p = 0.0017). In addition, cycle cancellation rate was significantly higher on PR group (4.0% vs 23.5%; p = 0.0128). This finding was confirmed through the logistic regression model (OR: 7.38, CI: 2.35–23.24; p = 0.016).

**Conclusions**

Embryo multiple abnormalities occurrence risk is more than 2-fold higher in PR patients. In addition, odds of cycle cancellation rate were more than seven-fold higher due to the decreased presence of normal embryos on PR group. Similar implantation and pregnancy rates in NR and PR groups could possibly be explained by equivalent embryo morphology and chromosomal status of transferred embryos after PGS performance. Poor ovarian response impairs embryo chromosomal integrity and percentage of viable embryos, reducing the chances of IVF cycles completion, and highlights the predictive value of aged women ovarian reserve for oocyte quality and competence.

**Funding** none.

**FC 7-4 Ovarian stimulation**

*Ovarian response and safety after exposure to corifollitropin alfa in repeated GnRH antagonist ovarian stimulation cycles*

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**Introduction**

A single injection of corifollitropin alfa, a new recombinant fertility hormone, is able to initiate and sustain follicular growth for an entire week. The Trust trial investigated the pharmacodynamics and safety of repeated exposure to corifollitropin alfa in patients undergoing up to 3 COS cycles.

**Materials and Methods**

*Women ≥ 18 and ≤ 39 years, with BMI ≥ 18 and ≤ 29 kg/m², weighing > 60 kg were included in this open-label, uncontrolled repeated-cycle study. 682 patients started with the first COS cycle, 375 continued with a second cycle and 198 with a third cycle. Each cycle started with a single dose of 150 µg corifollitropin alfa (Elonva, N.V. Organon), and when required, continued with daily rFSH from the 5th to the 17th day of stimulation, (as soon as 3 follicles > 17 mm). At day 5 or 6 of stimulation, patients started GnRH antagonist treatment up to and including the day of hCG criterion (as soon as 3 follicles ≥ 17 mm). At day 5 or 6 of stimulation, patients started GnRH antagonist treatment up to and including the day of hCG. About 34–36 h after hCG administration, oocyte pick-up (OPU) followed by IVF or ICSI was performed. At embryo transfer, 3 or 5 days after OPU, a maximum of 3 embryos were transferred. Patients were monitored for hypersensitivity reactions and possible antibody formation against corifollitropin alfa in each COS cycle.*

**Results**

Number of follicles ≥ 11 mm induced by corifollitropin alfa was similar between cycles 1, 2, and 3, resulting in a mean (SD) number of 14.5 (6.4), 13.9 (6.0), and 13.7 (6.4) follicles, respectively, on the day of hCG. Accordingly, mean (SD) number of oocytes retrieved was consistent with 11.9 (7.2), 11.5 (6.8), 11.3 (7.6), respectively. With a mean span of 10.0 days, total duration of stimulation was identical in these 3 cycles. In patients with 3 COS cycles, the mean (SD) number of follicles ≥ 11 mm was also similar between the cycles: 13.0 (6.1), 12.9 (5.6), and 13.7 (6.4), respectively. In cycle 1, incidence of moderate OHSS was 0.9% and severe OHSS was 0.9%, whereas in cycle 2 these incidences were both 0.5%. No cases of OHSS were reported in cycle 3. No adverse reactions related to immunogenicity were noted in any of the patients receiving 1, 2, or 3 injections of corifollitropin alfa.

**Conclusion**

Repeated exposure to a single dose of 150 µg corifollitropin alfa resulted in a similar ovarian response in each of the 3 COS cycles and was safe, with a low incidence of OHSS and no concerns related to immunogenicity.

**Support**

Financial support for this study was provided by Schering-Plough Corporation, now Merck & Co., Inc., Whitehouse Station, NJ, USA.

**FC 7-5 Ovarian stimulation**

*Quinagolide reduces OHSS in high risk ICSI patients*

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**Introduction**

Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening iatrogenic complication of controlled ovarian hyperstimulation. Severe forms of OHSS appear in 0.5–5% of assisted reproduction technology cycles. OHSS appears to be induced by the ovarian release vascular endothelial growth factor (VEGF) and its receptor 2 (VEGFR2), causing increase vascular permeability (VP). Dopamine agonist (DA) inhibit VEGF/VEGFR2 and thereby decrease VP.

**Aim of Study**

To determine the efficacy of non-ergot derived Dopamine agonist Quinagolide (Norprolac: Ferring Pharmaceuticals) in preventing OHSS in high risk women undergoing ICSI, and how would it affect the outcome?

**Materials and Methods**

*This randomized control prospective study was performed in the period from 1/6/2007 – 1/6/2009. The study included 198 women undergoing ICSI and at high risk of developing OHSS. F, level on day of hCG < 4000 pg/ml ≤ 20 follicles < 10 mm Patients were similar as regards age, cause of infertility (male factor), and BMI All patients were on long protocol using GnRH agonist. On day of HCG the patients were randomly divided into 2 groups: Group 1 (Study group: 100 patients): were given Quinagolide (Norprolac) 150 mg daily*
from the day of HCG administration for 15 days. Group 2 (Control group: 98 patients): were not given Quinagolide.

- Ultrasound guided embryo transfer (ET) of 2–3 embryos was performed after 46–48 hours.
- Luteal phase support was achieved with 400 mg vaginal micronized progesterone twice daily till day of pregnancy test.
- OHSS symptoms were assessed according to Golan’s classification system 4, 8 and 12 days after HCG administration.
- Ascites was determined by transvaginal ultrasound.

Results The incidence of OHSS in group 1 was 5%, while it was 20.4% in group 2 (p-value: 0.03) and all cases in group 1 were early onset OHSS. The clinical pregnancy rate was similar in two groups.

Conclusions The present study indicates that Quinagolide (Norprolac) is effectively reduced the development of OHSS in high risk patients, without affecting the clinical pregnancy rate.

FC 7-7 Ovarian deficiency “Effect of genital tuberculosis on ovarian reserve”, a retrospective analysis in patients of genital tuberculosis undergoing IVF

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Objective To evaluate the association of genital tuberculosis (GTB) with premature ovarian failure (POF) and poor ovarian response in young patients.

Methods GTB is a known cause infertility due to affection of tubes and endometrium, but due to alarmingly high rate of POF and poor ovarian response in young patients (<35 years) with GTB presenting for IVF at our clinic we decided to perform retrospective analysis to ascertain whether tuberculosis also affects the ovarian function. We analyzed 80 patients less than 35 years of age with regular periods who underwent IVF or ICSI between Jan 2005 till Jan 2008. Group A consisted of 40 patient with GTB while group B consisted of 40 patients of unexplained or male infertility. Results were compared in terms of Baseline (D-3) FSH value, No. of COC’s (Cumulus oocyte complex) recovered, oocyte quality & pregnancy rate.

Results 4 patients (10%) in group A had high basal FSH value (>10 IU/L) compared to 1 patient (2.5%) in group B. Total number of COC’s recovered in group A were 165 (average 4.125/pkt), compared to 354 (average 8.85/pkt) in group B. In terms of oocyte quality also oocytes recovered from the GTB patients showed poor quality. The clinical pregnancy rates in group A was 20% (8 patients) compared to 32.5% (13 patients) in group B.

Conclusions Patients with GTB show an overall poor ovarian response signifying effect on ovarian function.

FC 7-8 Ovarian deficiency Poor responder and dehydroepiandrosterone DHEA – when? how? and success

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Introduction To present the possible positive effect of dehydroepiandrosterone administration in assisted reproduction and especially in poor responders, women with diminished ovarian reserve, premature ovarian failure and premature ovarian aging in the course of ovarian stimulation protocols followed either by intrauterine insemination or IVF.

Materials and Methods We report the use 50–75 mg of dehydroepiandrosterone supplementation for at least 3 months in 150 women with confirmed diminished ovarian reserve, premature ovarian failure or premature ovarian aging. Positive effect has been reported to oocyte and embryo quality, even to women aged 40–47 years.

Pregnancies in natural or stimulated cycles It results in natural conception or considerably improves intrauterine insemination and IVF outcome and pregnancy rates and miscarriage rate is decreased. No significant side effects are reported, and those include mainly acne.

Conclusions The results obtained over the last few years confirm the improvement of oocyte production and pregnancy rates. More data on the dehydroepiandrosterone effect on assisted reproduction are needed.
FC 8-2 Infertility surgery
Comparative study between robotic laparoscopic myomectomy and abdominal myomectomy and factors affecting short-term surgical outcomes
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Study Objective To compare short-term surgical outcomes of robotic and abdominal myomectomy and to analyze the factors affecting those short-term outcomes.

Design Retrospective study of a consecutive case series.

Subjects and Method Between January 1992 and February 2007, 257 patients underwent abdominal myomectomy. Out of the 257 patients, 109 patients’ information was fully obtained. The patients’ data included: BMI, age, gravity and parity before and after myomectomy, the largest size of the leiomyoma, as it is a useful tool for checking the effect of new therapies for uterine leiomyoma.

Results No significant differences were found between the 2 groups regarding age, gravity and parity. However, the BMI was significantly higher in AM compared to RALM group (mean: 31.02 ± 7.15 kg/m² in AM vs 28.05 ± 5.98 kg/m² in RALM; p = 0.0155). The number of leiomyomata was significantly larger in AM compared to RALM group (average: 4.22 ± 3.36 in AM vs 3.06 ± 1.44 in RALM; p = 0.0094). The tumor size was significantly larger in AM compared to RALM group (mean: 53.11 ± 25.71 mm in AM vs 42.93 ± 17.91 mm in RALM; p = 0.0114). The total operative time was statistically longer in RALM compared to AM group (mean: 205.43 ± 73.58 min in RALM vs. 161.65 ± 49.99 min in AM; p = 0.0066). The total estimated blood loss was significantly lower in RALM compared to AM group (mean: 110.19 ± 62.48 ml in RALM vs. 176.11 ± 82.22 ml in AM; p < 0.0001). The length of hospital stay was shorter in RALM compared to AM group (mean: 1.08 ± 0.42 days in RALM vs 2.35 ± 1.24 days in AM; p < 0.0001) and the predicted odds of staying one day or less in the hospital for patients receiving RALM was 193.5 times the odds for patients receiving AM when adjusted for the number of leiomyomata and the tumor size. The total operative time was significantly increased by performing RALM rather than AM, higher number of leiomyomata, larger tumor size and increased parity. The estimated blood loss was significantly increased by AM rather than RALM, higher number of leiomyomata and increased tumor size. The probability of one day admission or less in the hospital was significantly increased performing RALM, decrease number of tumors, and decrease tumor size.

Conclusions RALM has shorter hospital stay, less blood loss and increased operative time compared to AM.

FC 8-3 Infertility surgery
Factors affecting recurrence of leiomyoma after myomectomy
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Study Objective To assess the factors affecting recurrence of leiomyoma after myomectomy.

Design Retrospective study of a consecutive case series.

Population 257 patients.

Intervention Abdominal myomectomy.

Subjects and Methods Between January 1992 and February 2007, 257 patients underwent abdominal myomectomy. The postoperative basal FSH levels did not change in comparison to the preoperative levels. There were smaller, but still significant, decreases in the serum AMH levels between to operation and was significantly reduced to a median level of 2.24 ng/mL at 1-month postoperation in the endometrioma group. There were smaller, but still significant, decreases in the serum AMH levels between pre- and post-operation in the non-endometrioma group (3.92 ng/mL to 3.29 ng/mL). The preoperative basal FSH levels did not significantly change in comparison to the preoperative levels. The results suggest that serum AMH levels, not basal FSH levels, could be a useful marker of ovarian reserve regarding laparoscopic ovarian cystectomy.

Results The xenografts fairly preserved their original histology. They significantly increased in size and proliferated upon treatment with E and P. We found that a certain cell density is required for the responsiveness of E plus P in tissue xenografts, and the growth failure of tissue xenografts due to the low cell density was restored by cell xenografts containing a certain number of cells. Immunohistochemistry revealed that ER was positive in all subgroups; in contrast, PR was only positive upon treatment with E and E plus P, suggesting that E induced PR through binding ER. Furthermore, RU486, a progestrone antagonist, strongly inhibited the enlargement of xenografts encouraged by E plus P. We have also succeeded in growing cell xenografts which GFP gene were transferred by a lentiviral system, and the xenografts retained green fluorescence upon treatment with E plus P.

Conclusions This model is suitable for understanding the role of E and P for the growth of uterine leiomyoma. We propose that xenografts become a disease model for uterine leiomyoma, as it is a useful tool for checking the effect of new therapies for uterine leiomyoma.
Results

In the cervical region CT-VHSG and the grade of discomfort were also documented. CT images were evaluated on a workstation (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula. CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.

Methods

We retrospectively evaluated 1500 CT-VHSG studies performed in our institution for the evaluation of infertility. The mean age of the studied population was 37.2 ± 3.9 years old.

CT-VHSG exams were performed using a 64-row CT scanner (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula. CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.

Results

In the cervical region CT-VHSG demonstrated: wall irregularities (23%), folds thickening (10%), cervical polyps (9%), diverticulae (6%), cervical stenosis (8%), cervical synechiae (1%). CT-VHSG findings in the uterus were divided according to the location. Uterine cavity findings were: polyps (40%), submucous myomas (9%), synechiae (11%). Uterine wall abnormalities included: intramural and subserous myomas (9%), uterine malformations (8%), adenomyosis (5%), and C-section scar (3%). Fallopian tubes findings included: unilateral hydrosalpinx (8%) and bilateral hydrosalpinx (2%), tubal obstruction (4%). The percentage of the fallopian tubes were partially visualized in the CT-VHSG studies. The scan time was 3.2 ± 1.1 sec. The effective radiation dose was 0.93 ± 0.08 mSv. The 86% of the patients referred only mild or no discomfort.

Conclusions

CT-VHSG allowed an adequate and accurate evaluation of the female internal genital organs, providing a complete diagnostic information in patients with infertility. This is minimally invasive low radiation dose technique, well tolerated for the vast majority of the patients. This modality appears as a valid alternative diagnostic test in the algorithm of patients with infertility.

Aims

The scanning time, the radiation exposure and the grade of discomfort were also documented. CT images were evaluated on a workstation (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula. CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.

Methods

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Results

In the cervical region CT-VHSG and the grade of discomfort were also documented. CT images were evaluated on a workstation (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula. CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.

Methods

We retrospectively evaluated 1500 CT-VHSG studies performed in our institution for the evaluation of infertility. The mean age of the studied population was 37.2 ± 3.9 years old.

CT-VHSG exams were performed using a 64-row CT scanner (Brilliance 64, Philips Medical Systems). Scans parameters were: 0.9 mm slice thickness, 0.45 mm reconstruction interval, 120 kV and 50-200 mAs. A total volume of 20 ml iodine contrast dilution (3ml of iodine contrast and 17ml of saline solution) were administered into the uterine cavity using a semi-rigid plastic cannula. CT images were evaluated on a workstation using multiple post-processing techniques. The scanning time, the radiation exposure and the grade of discomfort were also documented.
± 13.7 before treatment vs 18.7 ± 2.3 after treatment, Statistically significant increase in Hb concentration 7.3 ± 1.6 vs 13.2 ± 2.1 vs. after treatment.

Conclusions Ultrasound guided injection of OnKHFa in the treatment of leiomyoma is a new modality and new delivery system, reducing the cost of treatment, no effect on bone metabolism, no hypoestrogenic symptoms.

**FC 9: Menopause**

**FC 9-1 Menopause**

**Serum anti-Müllerian hormone levels change across the ovari-**

**latory menstrual cycle in late reproductive age**

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Anti-Müllerian hormone (AMH) has been used as a diagnostic marker to predict ovarian reserve in assisted reproductive technologies, premature ovarian failure, follow- ing cancer treatment and impending meno- pause. Its advantage is its circulating levels remain relatively constant over the menstrual cycle making it a more practical marker than other proposed tests.

The aim of this study was to explore the changes in serum AMH levels by ELISA across the ovulatory menstrual cycle from women in mid (n = 18) and late (n = 43) reproductive life in the menopause transition. Blood was collected three times a week across the menstrual cycle and its ovulatory status assessed based on the biphasic patterns of serum inhibin A, inhibin B, estradiol and progesterone.

No intracycle variation in AMH was observed in women in mid reproductive life (n = 18) nor in 33% (n = 14) of women with normal ovulatory cycles in late reproductive age (n = 43). In the remaining 29 cycles there was a significant 2-fold decrease (p < 0.01) in 11 cycles and a significant 4.2-fold increase (p < 0.01) in 10 cycles between the follicular and luteal phases. In a further 8 ovulatory cycles, AMH was below the level of assay detection. The failure to observe intracycle variation in AMH in women in mid reproductive life is consistent with published data. The detection of separate patterns of AMH in the follicular and luteal phases of ovulatory late reproductive age menstrual cycles presumably reflects the intermit- tent pattern of emerging follicles ap- proaching menopause.

These data indicate that a single AMH sample across the menstrual cycle is insufficient in the assessment of ovarian reserve in ovaries with reduced follicle number as observed in the menopause transition.

**FC 9-2 Sperm biology**

**Effect of testosterone enanthate on pro-**

**liferation and apoptosis of germ cells in damaged testis induced by busulfan**

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Background Chemotherapy or irradiation is associated with impaired fertility. The aim of this study was to investigate the possible protective role of testosterone enanthate (TE) on the proliferation activity, and apoptosis of germ cells in busulfan-induced spermi- toxicity.

Methods Sprague-Dawley rats were divided into 4 groups: The control group received vehicle (cotton seed oil) subcutaneously; Group 2 received A single doses of busulfan (15 mg/kg) intraperitoneally. Group 3 was administered two doses of (TE) 8 mg/kg subcutaneously, 21 days apart. Group 4 received both (TE) and busulfan. Half of animals were sacrificed on 5 days (short-term group) and the others on 52 days after treatment (long- term group) and evaluations were made by histological observations, bromodeoxyuridi- ne (Brdu) labeling, in situ TUNEL assay and measurement of serum testosterone levels.

Results Busulfan-treated rats both in short and long term groups, showed a significant increase in the percentage of apoptotic cells (p < 0.01). A significant increase in Brdu labeling index were observed in group 2 in long term animals (p < 0.001). However it was not significant in short term animals. Testosterone in group 4, significantly reduced rate of apoptosis compared to group 2 in short term and long term animals (p < 0.01). Administration of (TE) in group 4, significan- tly decreased Brdu labeling index in all animals, compared to group 2. A significant decrease in serum testosterone level was observed in the busulfan alone treated group of long- term treatment (p < 0.05). Administration of (TE) to group 4 significantly in- creased the levels of testosterone compared with busulfan alone both in short and long term animals (p < 0.01).

Conclusions Although the mechanism is not clear, these results indicated that Test- osterone enanthate may have a chemoprotective effect against busulfan-induced testic- ular damage, partly by decreasing of apoptosis and proliferative activity of germ cells.

Funding support for this study was provided by GUMS.

**FC 9-3 Menopause**

**Connection between estrogen re-**

**ceptors and pelvic organ prolapse**

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At present Pelvic Organ Prolapse remains a pressing problem in relation with emergence of new progress in science and revelation of new aspects in development of its pathology. Major work in this section is dedicated to implementation of new sling surgeries.

Application of monoclonal estrophillin anti- bodies and peroxidase-antiperoxidase tech- nique outline distribution of estrogen recep- tors in vagina, uterus and fallopian tubes of women in their reproductive years with nor- mal menstrual cycle. Principle mechanism of estrogen activity on reproductive organs con- cludes activation of dividing cell. Pelvic floor muscles would be sensitive to estrogen stimulation progressing during postmenopausal period independent of delivery or cae- sarean section.

Selective influence of steroid hormones on target tissue occurs in coordination with speci- fice cytoplasmic proteins. Hormone-protein complex affect at genetic level activating au- tologous protein cell which may manifest in form of cell proliferation, secretion of speci- fic substance and in particular receptors. Estrogen stimulates synthesis of receptors like progesterone. Progestins lowers concentra- tion of estrogen receptors.

Considerable estrogen effects on collagen metabolism of pelvic connective tissue have been noted boosting its synthesis and degra- dation. Replacement of old collagen with new one goes along with increase in cross- link numbers and it may outline strengthen- ing tissue in spite of general loss of collagen.

Extension of collagen fiber at vaginal wall, pararethral tissue and medium portion of levatory ani posterior passage allow it to control proximal part of urethra. In women with POP there’s marked drop in synthesis of col- lagen and substantial loss of amount of col- lagen on skin, round ligaments, vaginal epi- thelial and vesico-vaginal fascia. Collagen has long half-life and within some period at- tach to glucose-enzymatic glycation tissue. Initial product of this product may oxidize, form several components denoting the end product of glycation. Presence of these components reveals maturation of tissue. Monitoring decrease in end products of gly- cation, pentosidine after estrogen therapy supports hypothesis of rise in renewal of col- lagen.

Relevant evidence needs to be studied fur- ther, not just the distribution but comparative analysis of expression of estrogen receptors in women with Pelvic Organ Prolapse, that is being studied at present by the authors at the facilities of the Medical Center.
Introduction Use of hormone therapy (HT) has been associated with higher incidence of hormone receptor positive breast cancer in postmenopausal women. However, it is unclear if breast cancers developing after HT use are different in course of disease and prognosis from tumors growing without prior exogenous hormone use. This is the first study differentiating data on perimenopausal women from information on postmenopausal women with or without prior HT in diagnosed breast cancer patients.

Methods Women with hormone receptor positive breast cancer diagnosed between 1984 and 2006 at our university breast cancer center (n = 1248) were analyzed in a single-center retrospective trial. Perimenopause was defined by natural LMP ≤ 12 months or FSH/estriadiol in perimenopausal range for women after hysterectomy. Postmenopausal state was defined as natural LMP > 12 months preceeding diagnosis, high FSH and low estradiol in hysterectomized patients or by surgical menopause. Outcome data comparing overall survival and local and distant recurrence were extracted from the hospital cancer data base and the local breast cancer registry, as well as from primary care-giving gynecologists and by review of patient charts. Cases were stratified according to hormone therapy use before breast cancer diagnosis.

Results 1051 patients with hormone receptor positive non-metastasized invasive breast cancer were included in the final analysis. 201 (19.1%) women were premenopausal, 122 (11.6%) were perimenopausal and 728 (69.3%) were postmenopausal. Information on former HT use was available for 931 (88.6%) patients. 432 (41%) patients had never used HT, including 198 premenopausal patients. Out of 388 HT users 322 (83%) had never used HT, including 198 premenopausal patients. 432 (41%) patients had never used HT. Among these 7 patients were selected from 21 patients referred to our clinic because of delayed puberty between January 2006 and March 2009. In the selected subjects, height was ≤ 2SD and all of them had been receiving GH, but had never previously received ER. ER was administered with an adhesive transdermal patch containing 0.72 mg estradiol (E2). The initial dose was 1/8 of the patch and the dose was then increased to achieve the optimal serum E2 level. Height, Tanner stages, bone mineral density, serum E2 level, and uterine size were monitored every 3 months.

Results The mean serum E2 level was 9.2 ± 5.2 pg/ml (range 3.7–19.8 pg/ml) before the replacement therapy was initiated. The E2 dose was maintained at 1/4 of the patch in six cases and 1/2 in 1 case. The mean serum E2 level during the replacement was 13.8 ± 9.4 pg/ml (range 2.7–35.5 pg/ml). The bone mineral density increased in all cases (0.038 ± 0.021 g/cm² over 6 months). The mean height gain in patients who started replacement before age 15 was 2.77 ± 0.2 cm over a 30% increase in the rate of DNA fragmentation in samples showing high incidence of abnormal cells. Despite the benefits of IMSI in high DNA fragmentation patients, it could not be demonstrated on fertilization potential and embryo quality. The outcomes improvement observed after high-magnification could be explained by the later paternal effects on embryo development.

Funding none.
six months. Patients who initiated therapy after age 15 gained only 0.56 cm over 6 months. Uterine size increased in all patients, but there were no apparent changes in Tanner stages.

Conclusions Using a fractional piece of transdermal E2 patch is a useful approach that could increase both final height and bone mineral density without accelerating bone age in girls with ISS receiving GH. As the patients who started this therapy before the age 15 years gained more height than those who stayed after that age, we conclude that it is strongly recommended that low-dose ER should be initiated before 15 years of age.

Source of Funding none.

FC 9-7 Menopause
Ovulation and loss of bone density during the perimenopausal transition: The PeKnO-Study
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Introduction The PeKnO-Study is a prospective observational study on the influence of perimenopausal luteal phase hormones and ovulation patterns on bone mineral density (BMD) changes over 2 years. Computer-based cycle monitoring, pre-menstrual serum hormones, and BMD measurements by QCT are used to answer the question: Is there a correlation between decreasing rates of ovulation during the menopausal transition and increased perimenopausal bone loss?

Methods Cycle-length and ovulation were monitored by participants using a commercially available cycle-monitor. Serum hormones and bone-markers in bone density in perimenopausal women (aged > 45 years) during 2 years and analyzes the influence of hormonal factors on bone metabolism. Trabecular bone density (BMD) in lumbar spinal bones 1–3 was measured at the beginning and after 2 years using quantitative computer-tomography (QCT). All women monitored ovulation with a commercially available cycle-monitor. Every 6 months, hormone- and bone-markers in the luteal phase were measured, and cycle data transferred to a PC.

Results Out of 68 recruited participants, 5 study visits (0, 6, 12, 18, 24 months) were completed by 50 women. Results from 44 complete 2-year-courses are presented. Trabecular bone loss (mean -5.96 mgCa-HA/ml; SD 9.25) correlated with the rising FSH-levels. Modelling with Generalized Estimating Equations (GEE) showed a significant correlation between the change of BMD and FSH, with BMD decreasing by 1 mgCa-HA/ml for every 1 IU/1 increase of FSH; p = 0.001.

Conclusions FSH plays a role in the complex regulation of bone metabolism prior to the postmenopausal estrogen decline.

Reference:

FC 9-8 Menopause
Do elevated FSH-levels during perimenopause increase loss of bone density?
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Introduction Elevated FSH-levels are measurable a long time before menopause. Recently, a direct FSH action on resorption of bone mass was postulated [1]. Therefore, this study analyzed the influence of FSH on the change of bone density in perimenopausal women.

Methods The PeKnO-Study prospectively monitors menstrual cycles, hormone values, bone markers and bone density in perimenopausal women (aged > 45 years) during 2 years and analyzes the influence of hormonal factors on bone metabolism. Trabecular bone density (BMD) in lumbar spinal bones 1–3 was measured at the beginning and after 2 years using quantitative computer-tomography (QCT). All women monitored ovulation with a commercially available cycle-monitor. Every 6 months, hormone- and bone-markers in the luteal phase were measured, and cycle data transferred to a PC.

Results From 68 recruited participants, 5 study visits (0, 6, 12, 18, 24 months) were completed by 50 women. Results from 44 complete 2-year-courses are presented. Trabecular bone loss (mean -5.96 mgCa-HA/ml; SD 9.25) correlated with the rising FSH-levels. Modelling with Generalized Estimating Equations (GEE) showed a significant correlation between the change of BMD and FSH, with BMD decreasing by 1 mgCa-HA/ml for every 1 IU/1 increase of FSH; p = 0.001.

Conclusions FSH plays a role in the complex regulation of bone metabolism prior to the postmenopausal estrogen decline.

Reference:

FC 10: Cell biology

FC 10-1 Stem cells
Stem cells restored ovarian function and folliculogenesis in rats following induced ovarian failure
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Introduction Premature ovarian failure (POF) is a heterogeneous syndrome affecting 1% of women below the age of 40 years and 0.1% before 30 years. Infertility is a major problem that affects these women and currently no treatment is available that effectively increases fertility. Mesenchymal stem cells (MSCs) represent a promising tool for new clinical concepts in supporting cellular therapy.

Aim The aim of this experimental animal study was to explore the therapeutic potency of MSC transplantation for chemotherapy-induced ovarian damage in rats.

Materials and Methods This was a prospective case control experimental animal study. 60 mature female rats were studied. 15 rats served as a control group (group I). 45 rats were injected by intra-peritoneal cyclophosphamide (CTX). The study group was subdivided into 3 equal groups (group II, III and IV). Rats of group II were injected intra-peritoneally by male MSC, while group III by saline and group IV did not receive any injections. The rats were followed up for eight weeks by daily vaginal smear and biweekly E2 and FSH levels to monitor the ovarian activity. PCR was done to look for sry gene expression and Y chromosome incorporation into the ovarian tissues. Two rats were sacrificed every 2 weeks for histopathological examination for the ovarian tissues.

Results Ovarian failure was achieved in the study group by two weeks. The hypoestrogenic and hypergonadotropic state was reversed in the group that received MSC injection by the 8th week. There was no statistical difference between group 1 and 2 after 8 weeks of follow up as regards the mean serum FSH (3.60 ± 0.08 mIU/mL vs 5.38 ± 0.31 mIU/mL; p = 0.1, respectively) and E2 levels (69.71 ± 1.26 vs 53.5 ± 0.93 pg/mL; p = 0.2; respectively). Also, cytological and Histopathological examinations showed re-creation of ovarian folliculogenesis and corpus luteum formation in group II and such changes were not observed in the other groups. The (sry) gene expression of the Y chromosome was detected within the ovarian tissues in group II.

Conclusions Stem cells have the power of recovering ovarian function both in its hormonal and follicular development abilities. Our work has proved that this principle is achieved mainly by incorporation of stem cells into the ovarian structure and not merely by the paracrine effect.

Materials and Methods A total of 348 patient treatment cycles undergoing IVF/ICSI were included in this study. Exclusion criteria for this study included patients having PGD/PGS, elective complete cryopreservation and fertility preservation cycles. Embryos were allocated to either low-oxygen incubators at 37°C in 5% CO2/5% O2/90% N2 or to high-oxygen incubators at 37°C using 5% CO2 in air. The clinical endpoints were the rates of implantation (IR), embryo development and clinical pregnancy rate (CPR) per transfer. Outcome parameters were compared statistically using chi square analysis.

Results There was no statistical difference in CPR for patients ≤ 40 years of age between the treatment groups (46.0% CPR in low oxygen, n = 126 vs 43.7% CPR in the high oxygen group, n = 158; p-value = 0.69). No difference was observed in IR in the low oxygen group (25.3%) compared to the high oxygen group (24.6%, p-value = 0.84). In patients > 40 years of age, no difference was observed in CPR or IR (25.0% CPR, 9.8% IR, n = 24) in the low oxygen group vs (37.5% CPR, 12.1% IR, n = 40) the high oxygen group (p-value = 0.30 and 0.59). No difference in embryo quality was noted in patients ≤ 40 years of age for either group. Patients > 40 years of age in low oxygen tended toward better quality embryos with a higher cumulative embryo score (CES = 7.54) and more embryos developed beyond 5 cells (73.6%) compared to the higher oxygen group (CES = 7.02) with 63% of embryos growing beyond 5 cells (p-value = 0.039).

Conclusions Culturing early stage embryos in a low oxygen environment did not significantly improve clinical outcome parameters. Previous studies have shown significant improvement in clinical outcomes with later stage embryos cultured under low oxygen conditions. This suggests the possibility there is a critical point when low oxygen has a positive impact on outcome. While culturing early stage embryos in either low or a high oxygen environment seems like a viable option, it is not known if other detrimental effects occur when embryos are cultured in atmospheric oxygen. Further studies need to be performed to fully evaluate the effects of oxygen concentration in relation to gene expression and oxidative stress on the culture of human embryos.
sperm injection (ICSI) using nano ultra-high pressure chromatography (nanoUPLC) and mass spectrometry (MS).

**Materials and Methods** 17 embryos from 8 patients undergoing ICSI were split according to the implantation rate outcome: positive (n = 9) and negative (n = 8). After embryo transfer, the remaining culture media were collected and samples were pooled, adjusted to final protein concentration of 1 µg/µL and enzymatically digested with trypsin. NanoUPLC tandem nanoESI with MS² acquisitions were performed with a Qq-IMS-oxTOF Synapt HDMS™ (Waters, Manchester, UK). Quantitative data package were generated with ProteinLynxGlobalServer v.2.4 Expression® software and the UniProtKB/Swiss-Prot database. Only proteins in attendance scores and confidence higher than 99% were considered in order to accept these database searches.

**Results** It was observed unique proteins secreted by the embryo in each group. Jumonji, a nuclear protein from histone methyltransferase complex, was only observed in the positive-implantation group. This protein modifies methylation, acting as a negative regulator of cell proliferation signaling. Exclusively at the negative-implantation group, there was a release of TSGA10, a perinuclear protein which has structural activity and occurs in actively dividing and fetal differentiating tissues. Thus, it may be involved in active cell division and differentiation.

**Conclusions** Competent embryos have a differential protein expression, with some proteins secreted into the surrounding culture medium being potential biomarkers. Embryo viability has been associated with a quiet rather than active metabolism, as RNA transcripts are more frequently up-regulated in oocytes and embryos before the embryonic genome activation presenting lower developmental potential. This pattern may also be related to protein function, since Jumonji protein, only observed at positive-implantation group acts as a gene expression repressor. Otherwise, TSGA10 protein, whose detection was only at negative-implantation group, enhances gene expression. Secretome analysis may assist embryo selection based on morphological assessment in revealing secreted factors that reflect developmental competence and viability, and could predict a successful pregnancy, leading to single embryo transfer.

**Funding** none.

**FC 10-6 Embryo**

**The effects of antioxidants and feeder cells on developmental rate and blastomers number of mouse embryos exposed to sub-optimal conditions**

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**Objective** In laboratory embryos are inevitably exposed to different doses of visible light that could interfere with developmental capacity of embryos. In this study we compared the effect of 2 antioxidants, vitamin c and beta-mercaptoethanol, and human umbilical cord matrix cells in removing the deteriorating effects of visible light on mouse 2-cell embryos.

**Materials and Methods** 2-cell mouse embryos were exposed to 1600 lx visible light for 30 min. A non-exposed group served as control. Embryos in control group were transferred to MEM-alpha supplemented with FBS and exposed embryos transferred to two groups comprise the same media plus antioxidants and feeder cells. Developmental rate was assessed for 96 h. At this time point, some of the embryos in 3 groups were randomly selected for differential staining with Hoechst and PI and the rest were monitored for another 24 h.

**Results** Exposed embryos treated with antioxidants and human umbilical cord matrix cells showed significant difference (p < 0.05) in blastomers number count when compared to non-exposed embryos cultured in conventional conditions and also in developmental rate there was a significant difference (p < 0.05) between exposed embryos in co-culture group and non-exposed embryos.

**Conclusion** Antioxidants and feeder cells are probably capable of removing free radicals from embryo culture media. This property may shorten the cell cycle interval and result in higher cell number in embryos.

**FC 10-7 Stem cells**

**Evaluation of protective effects of different feeder layers on mouse embryonic stem cells**

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**Application of stem cells in medical research and treatment has made a new horizon to progress in many disorders. So finding methods for better isolation and culture and maintenance of Sc has a specific importance. Aim of this reach was to evaluate of protective effects of 3 different layers of MSC, MEF and 3T3 on Sc for finding the best feeder layer for increasing the amounts of sells for transplantation and differentiation. Till to access an applied and easier method for Sc in treatment.**

**Purpose** Evaluation of protective effect of MEF, MSC and 3T3 feeder layers in isolation and culture of mouse embryonic stem cells.

**Blastocysts** were obtained from Balb/c pregnant mice. Collected embryos were put on 3 different feeder layers of mouse embryonic fibroblast (MEF) Mesenchymal stem cells (MSC) and 3T3 cell line. 2 or 3 days later, the zona pellucida was removed. And after 5 days the inner cell mass (ICM) growth, was removed mechanically and changed to small multi-cellular clumps through trypsinization. Colonies of stem cells were formed after 2 to 3 days. This repeated to two other passages and each time, the amount of colonies formation was increased. Then formed colonies were cultured on feeder layers. Finally the colonies were identified through staining with alkaline phosphates for their numbers and morphological characteristics.

**Findings** From 29 embryos put on MEF layer all of them (100%) was attached. From 32 embryos put on the MSC layer (64.5%) was attached. While the amount of attaching of 20 blastocyst to the 3T3 feeder layer was law and none of them reach to the growth stage of the inner cell mass. The amount of colonies formation during three passages on MEF feeder layer was more than MSC feeder layer. The colonies were identified through staining with alkaline phosphates. Data analysis demonstrated a significant difference (p < 0.05) in percentage and time of hatching and time for living of blastocysts.

**Conclusions** Considering these 3 feeder layers, MEF was better than MSC and both of them had priority to 3T3.
Methods 36 patients underwent routine endometrial biopsy during diagnostics prior to infertility therapy. Endometrial stroma cells were isolated by immunodepletion and the expression of stem cell markers was analyzed by quantitative real time PCR and FACS analysis. Differentiation potential and clonogenic efficiency of serially passaged cell clones were studied in vitro.

Results Endometrial stroma cell clones derived from endometrial biopsies displayed characteristic properties of stem cells including clonality, long time cultivation, multipotent differentiation potential and expression of the stem cell markers CD146, CD73, Msi-1, Notch-1 and Sox-2.

Conclusion Adult stem cells can be obtained from endometrial biopsies in a routine diagnostic setting. These findings will stimulate and accelerate stem cell based studies to develop innovative diagnostic and therapeutic concepts of endometrial disorders in large patient populations.

FC 11: Implantation

FC 11-1 Implantation

Identification of novel proteins associated with uterine receptivity in fertile women

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Molecular mechanisms which operate in spatio-temporal manner to endow the endometrium with receptivity are not yet elucidated in humans. This may be attributed to our incomplete knowledge of the key players, which are of crucial relevance in endometrial receptivity. In the present study a proteomics based approach was adopted to generate a catalogue of the major secreted and cellular proteins expressed during the proliferative and mid secretory/receptive phase in fertile women. Women of reproductive age with a history of regular menstrual cycles were enrolled in the study. Uterine fluid samples collected during the mid secretory phase (n = 6) and proliferative phase (n = 5) were resolved by two dimensional gel electrophoresis in the linear range of pI 4–7. Fifteen protein spots found differentially expressed between the two phases. Immunohistochemical analysis was performed to confirm the inferences drawn from the densitometric analysis of the 2D gels. Our study is the also the first to report cycle dependent variations in the expression of endometrial calreticulin. The present study offers valuable clues regarding the differential expression of novel as well as some already known proteins expressed during the mid secretory/receptive phase of the endometrium. Some of these identified proteins could be analyzed further to be proposed as biomarkers to distinguish the receptive and non receptive state of the endometrium.

FC 11-2 Implantation

The expression of natural cytotoxicity receptors on natural killer cells from midsecretory endometrium and aborted decidua

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Objectives Natural cytotoxicity receptors (NCRs) are unique markers, which regulate NK cell cytotoxicity and cytokine production. In this study, we aimed to investigate the expression of NCRs on NK cells in the endometrium and aborted decidua from women with implantation failures or recurrent pregnancy losses (RPL).

Method of Study The expression of NCRs (NKp46, NKp44 and NKp30) on NK cells (CD56<sub>dim</sub> and CD56<sub>bright</sub>) in the endometrium and the aborted decidua was analyzed using 3-color flow cytometry. NK cell subpopulation (CD16 and CD56) in the endometrium and the aborted decidua was also evaluated using same method. NK cells from the midsecretory endometrium (19 endometria from women with implantation failures, 9 from women with PRL, and 40 from controls) were collected by using endometrial sampler, while those from aborted decidua (n = 11) were collected by dilatation and curettage. Endometrial and aborted decidual samples were mechanically dispersed using a tissue grinder.

Results The aborted decidua had significantly higher percentages of CD16<sup>+</sup>/CD56<sup>dim</sup> cells, CD56<sup>dim</sup>/NKp44<sup>+</sup> cells, and CD56<sup>dim</sup>/NKp30<sup>+</sup> cells than the midsecretory endometrium. NKp46 (p < 0.001), NKp44 (p < 0.001) and NKp30 (p < 0.001) were significantly expressed on midsecretory endometrial CD56<sup>dim</sup> cells than those of CD56<sup>dim</sup> cells, while in the aborted decidua only NKp46 was up-regulated on CD56<sup>dim</sup> cells than that of CD56<sup>dim</sup> cells. On the other hand, in women with implantation failures, the expression of NKp46 (p < 0.001), NKp44 (p < 0.05) and NKp30 (p < 0.001) on endometrial CD56<sup>dim</sup> cells were significantly up-regulated as compared with those of CD56<sup>dim</sup> cells. However, in women with RPL and controls, expression of NKp30 (both p < 0.01) did not change. NKp44 (p < 0.001 in RPL, p < 0.05 in controls) on endometrial CD56<sup>dim</sup> cells were significantly augmented than those on CD56<sup>dim</sup> cells, but no difference in expression of NKp44.

Conclusions The different profile of NCRs expression in endometrial and aborted decidua NK cells may suggest presence of abnormal regulation of NK cell in women with reproductive failures. NKp44 is expressed on the surface of activated NK cells only. Consequently, the differential expression of NKp44 in women with implantation failures, namely higher NK cell cytotoxicity exists. This study was supported MEXT KAKENHI (21791536).

FC 11-3 Implantation

The expression of natural cytotoxicity receptors and the NK cell cytokines production in pregnant women with a history of recurrent pregnancy loss and pregnancy-induced hypertension

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Objectives Natural cytotoxicity receptors (NCRs) are markers which regulate NK cell cytotoxicity and cytokine production. The aim of this study was to investigate the expression of NCRs and intracellular cytokine expression of peripheral blood NK cells in pregnant women with a history of recurrent pregnancy losses (RPL) and preeclampsia. We also aimed to clarify the correlation between NCRs and intracellular cytokine expression of NK cells in pregnant women.

Method of Study The expression of NCRs (NKp46, NKp44 and NKp30) and cytokines (TNF-α, IFN-γ, IL-4, IL-10) in peripheral blood NK cells (CD56<sup>dim</sup> and CD56<sup>bright</sup>) was analyzed using flow cytometry in pregnant women with a history of RPL (n = 13) and preeclampsia (n = 10). The blood samples were collected from these subjects at 12, 20, 28 and 36 gestational weeks. Blood samples were also collected from pregnant women with no history of RPL and preeclampsia, and were served as control.

Results Pregnant women with a history of RPL had significantly decreased percentages of CD56<sup>dim</sup>/NKp46<sup>+</sup> cells (p < 0.001) and CD56<sup>dim</sup>/NKp30<sup>+</sup> cells (p < 0.05) than those of control. There was no significant difference in the percentage of NKp44 expressing NK cells between pregnant women with/ without a history of RPL. Pregnant women who had a preeclampsia showed significantly decreased percentages of CD56<sup>dim</sup>/ NKp46<sup>+</sup> cells (p < 0.01), and CD56<sup>dim</sup>/NKp46<sup>+</sup> cells (p < 0.01) than pregnant
women without preeclampsia. There were no differences in the percentages of NKp30 and NKp46 expressing NK cells between pregnant and non-pregnant women. For type 1 cytokine expressing cells, CD56+/NKp46+ cells in pregnant women were negatively correlated with CD56<sup>dim</sup>/IFN-γ cells (r = 0.495; p < 0.02) and positively correlated with CD56<sup>bright</sup>/IFN-γ/TNF-α cells (r = 0.516; p < 0.02). For type 2 cytokine expressing cells, there were no correlations between NCRs and intracellular cytokine (IL-4 and IL-10) expression of NK cells.

Conclusions Women with a history of RPL and preeclampsia carry immunological abnormalities of NCRs on peripheral blood NK cells during the course of pregnancy. Evaluation of NCRs, especially NKp46 on peripheral blood NK cells may be useful for prediction of preeclampsia. The low expression of NKp46+ NK cells in women with preeclampsia may account for the high production of NK1 cytokine that is known as NK1 shift in pregnant women with preeclampsia.

This study was supported by MEXT KAKENHI (21791536).

FC 11-4 Implantation
Effect of tissue specific Connexin26-deletion in the uterine epithelium on embryo implantation

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A precise molecular interaction between the blastocyst and the receptive uterine endometrium is indispensable for successful implantation. Disruption of this interaction leads to failure of implantation. We could show before that the gap junction Connexin(Cx)26 is precisely regulated during early pregnancy and is induced prior to implantation by the blastocyst in the luminal epithelium of the implantation chamber in rodents. However, the role of this specific induction for embryo implantation in not known yet.

Since Cx26-knockout mice are not viable due to a placental defect, we here generated mice with an endometrium-specific Cx26-deletion by using the Cre/lox-system. Female mice homozygous for Cx26<sup>−/−</sup> and heterozygous for the Cre-recombinase gene under control of the Pax8-promoter which is expressed in the endometrial epithelium were generated (Pax8-Cre<sup>−/−</sup>-Cx26<sup>−/−</sup>). Deletion of Cx26 in the uterine epithelium was verified by qPCR and immunohistochemistry. Female Pax8-Cre<sup>−/−</sup>-Cx26<sup>−/−</sup>- mice as well as controls without the Cre-recombinase gene were mated with wild-type males and litter sizes were analyzed. In addition, on 4.5, 5.5, and 6.5 dpc implantation chambers were examined histologically.

The tissue specific deletion of Cx26 in the uterine epithelium lead to a significant reduction of litter sizes compared to controls. In parallel, an increase in embryonic degeneration could be observed on 5.5 dpc in the implantation chambers of Pax8-Cre<sup>−/−</sup>-Cx26<sup>−/−</sup>/mice. The signals cascades affected by this endometrial Cx26 deletion are under investigation.

FC 11-5 Implantation
Suppression of CD16 CD56<sup>−</sup> NK cell subset in the early secretory endometrium in patients with or without endometriosis

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Several reports indicate that NK cell activity has a key role in the implantation process in patients with or without endometriosis. For example, it has been suggested that NK cell activity is inhibited in patients with endometriosis. It is also known that patients with abnormal NK subpopulation such as higher ratio of CD16<sup>−</sup>-CD56<sup>dim</sup>/CD16<sup>−</sup>-CD56<sup>bright</sup> in endometrium have higher risk towards abortion. This study was conducted to know the distribution in the NK cell subpopulation in the eutopic endometrium throughout the menstrual cycle in patients with or without endometriosis.

The subjects were consisted of patients with controls (n = 92) or endometriosis (n = 51) with regular menstrual cycles. Each endometrium was obtained for the analysis of NK cell subpopulation using 2-color flow cytometry. Obtained endometria were dated according to the criteria by Noyes et al. Data were analyzed using one-way analysis of variance (ANOVA) or unpaired t-test.

In controls or endometriosis, CD16<sup>−</sup>-CD57<sup>−</sup> cell subsets did not vary during the menstrual cycle. Among CD16<sup>−</sup>-CD57<sup>−</sup> cell subset, CD16<sup>−</sup>-CD56<sup>−</sup> cells varied drastically during the cycle. That is, the number of cells was highest (49.4, 47.2) in the early proliferative phase, and lowest (13.1, 15.3) in the early secretory phase (p < 0.0001). The suppression was reached to 26.5 or 32.4% of that in the early proliferative phase, respectively. The number in the endometriosis group showed a similar pattern with controls. Of particular interest was the change in CD16<sup>−</sup>-CD56<sup>−</sup> cells. That is, the variation was similar to those in CD16<sup>−</sup>-CD56<sup>−</sup> cells (p < 0.0001), while CD16<sup>−</sup>-CD56<sup>−</sup> cells did not vary throughout the cycle. In contrast, CD16<sup>−</sup>-CD56<sup>−</sup> cells revealed an asymmetric pattern against CD16<sup>−</sup>-CD56<sup>−</sup> cells. That is, the number increased towards the ovulatory phase, peaked in the early secretory phase, and reduced towards the menstruation. The number in the endometriosis group showed a similar pattern to controls.

In conclusion, CD16<sup>−</sup>-CD56<sup>−</sup> cells or CD16<sup>−</sup>-CD56<sup>−</sup> cells varied drastically during the menstrual cycle and reached the nadir in the early secretory phase in controls as well as endometriosis. It became obvious that those NK cell subsets have a key role in achieving pregnancy. However, it is thought that endometriosis itself might not adversely affect fecundity as with immune cell system in the endometrium.

FC 11-6 Implantation
Assessment of uterine receptivity by endometrial blood flow in women with and without tuberculosis undergoing IVF-ET in a developing country: A prospective observational study

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Aim This prospective observational study was conducted at AIIMS, New Delhi to find the answer to the question whether endometrial blood flow using colour Doppler as a tool in the women with history of Tuberculosis was different from the one who did not had tuberculosis in the past.

Materials and Methods A total of 183 infertile women were recruited from our IVF-ET programme from January to December 2009. On the day of HCG administration sub endometrial and endometrial blood flows, PI and RI of the uterine artery was examined by the same observer. Power Doppler was placed over the entire endometrial and sub-endometrial areas in this longitudinal section. We applied the same power Doppler characteristics like normal quality of colour, colour gain −3.4, pulse repetition frequency of 600 Hz, and wall motion filter of 50 Hz. The plane with the maximum flow was considered standard. The maximum endometrial thickness was defined as the greatest distance between both the mayo-endometrial interfaces in the longitudinal plane.

Statistical Analysis Statistical analysis was performed for continuous variables using student’s t test and categorical data was analyzed using Fischer’s exact test or Chi² test; p < 0.05 was considered significant.

Results In our study 88 (48.1%) had taken Anti Tubercular Treatment (ATT) in the past for genital or extra-genital tuberculosis (TB). The indications were pulmonary or abdomino-nal Koch’s, Laparoscopy or hysterosalpingography (HSG) findings, TB endometritis, Salpingitis, lymphadenopathy, Pott’s spine, genitor urinary TB and a positive PCR. The mean Gonadotropin usage in Group 1 was 2881.3 IU (Mean ± SD 949.7) and 3077.8 IU (Mean ± SD 927.9) in Group 2 (95%-CI: 473.43–80.42; p = 0.16). Total number of eggs obtained, duration (days) of stimulation, Estradiol (E2) concentration and endometrial thickness (in mm) showed no significant difference between the two groups.

In the answer to the question whether endometrial blood flow using colour Doppler as a tool in the women with history of Tuberculosis was different from the one who did not had tuberculosis in the past.

The mean values for Peak Systolic velocity, Pulsatility Index (PI) and Resistance Index (RI) was not statistically
**FC 11-7 Embryo**

**Correlation between zona pellucida thickness and morphological characteristics of human embryos in IVF treatments**

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**Introduction**
Assessment of embryo morphology is of great importance in predicting embryo viability and selecting the best embryos for transfer. In addition to the conventional embryo characteristics, thickness and morphology of zona pellucida (ZP) may also have an important role in the embryo selection process. ZP characteristics can also predict the necessity of assisted hatching. In our recent study we analyzed the correlation between ZP thickness and morphological characteristics of human embryos. Effects of ZP thickness on further embryo development were also examined.

**Materials and Methods**
In our retrospective study we analyzed 3156 embryos which were transferred in 1194 IVF treatment performed between November 2005 and December 2009. Besides conventional embryo grading, ZP thickness was also measured on every transferred embryo. Average ZP thickness of digital embryo images was calculated using OCTAX Eyeware computer program. Transferred embryos were classified into three groups according to their average ZP thickness as follows: Group A: < 15 µm (Thin); Group B: 15–20 µm (Normal); Group C: ≤ 20 µm (Thick). Morphological features of embryos like cell number, amount of fragmentation and morphology score were compared between groups. Correlation between ZP thickness and embryo implantation were analyzed in those IVF cycles where all embryos were implanted or none of the embryos were implanted.

**Results**
Average cell number in embryos was significantly lower in Group C than in Group B (6.2 ± 2.3 vs 5.9 ± 2.3; p < 0.01). The amount of fragmentation was the highest in the thick ZP group (A: 15.6 ± 9.6; B: 16.4 ± 9.4; C: 17.6 ± 10.8) and the difference was significant (p < 0.01). Average morphology score was lower in the thick ZP group (A: 2.25 ± 0.51; B: 2.27 ± 0.5; C: 2.21 ± 0.52) and the difference was significant (p = 0.03).

**Conclusions**
Zona pellucida thickness seems to be related to some of the morphological characteristics of the embryo, such as the cell number, amount of fragmentation or morphology score, which can predict the embryo viability. Embryos with thick zona pellucida have a poorer embryo development and a lower morphology score. However, we could not clearly demonstrate the unfavourable effect of thick zona pellucida on further embryo implantation.

**FC 11-8 Pre-implantation embryo culture systems on the probability of pregnancy: A randomized controlled trial**


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**Introduction**
Embryo culture represents an integral part of the IVF process and is an important determinant of success. The aim of this study was to evaluate the effect of using two different embryo culture systems on the probability of pregnancy in patients undergoing IVF.

**Materials and Methods**
From January 2009 until November 2009 all patients treated by fresh IVF/ICSI (n = 692) with at least one cumulus oocyte complex (COC) retrieved were randomly allocated to 2 treatment groups. In Group A (n = 343), the full SAGE culture system was used, while in Group B (n = 349), a mixed system consisting of Medicult™ media up to the fertilization point followed by the Vitrolife G5 series™ media for the remaining embryo culture days, were used.

The primary outcome measure was clinical pregnancy rate per randomized patient. Furthermore, results were compared in terms of fertilization rates, positive human chorionic gonadotrophin (hCG) and embryo quality.

Embryo quality was compared by using a cumulative embryo score (CES), which was calculated for each patient, for a given day (day 2 and day 3 of culture) by summing the quality scores of all embryos present on that day. Embryo quality score was assessed by multiplying embryo grade score by the number of blastomeres present in that embryo. Grade 1.0 and 2.0 embryos were given a grade score of 4, grade 2.1 embryos were given a grade score of 3, grade 2.2 embryos were given a grade score of 2, while grade 3.1–3.2 embryos were given a grade score of 1.

**Results**
No significant differences were noted between groups in terms of female age, total number of COCs retrieved. Fertilization rates were significantly different between Group A and Group B (59.96% ± 22.48 vs 54.37% ± 22.88, respectively; p = 0.003).

**Conclusions**
The CES for days 2 and day 3 of embryo culture were not significantly different between the 2 groups compared.

The mean number of embryos transferred was significantly different between the 2 groups compared (Group A: 2.53 ± 0.81 vs Group B: 2.39 ± 0.78; p = 0.03). Clinical pregnancy was significantly increased in Group A as compared to Group B (Group A: 34.4% vs Group B: 25.2%; rate difference: +9.2%; p = 0.01) rates per randomized patient.

**FC 12: Ovulation induction**

**FC 12-1 Polycystic ovaries**

**Polycystic ovary syndrome is associated with a decreased level of endothelial progenitor cell count**

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**Objective**
Endothelial progenitor cells (EPCs) have been reported to be associated with the risk of cardiovascular disease, metabolic syndrome and insulin resistance. However, the relationship of endothelial dysfunction to polycystic ovary syndrome (PCOS) remains unclear. Hence, the purpose of this study was to determine the endothelial progenitor functions of PCOS patients by measuring circulatory EPCs.

**Materials and Methods**
Circulatory EPCs were accessed by measuring the percentage of CD146+CD34+CD133+ and CD34+CD184+ cells using fluorescence activated cell sorting (FACS) analysis in peripheral blood samples of a total of 47 PCOS patients.

**Results**
Compared with normal population, our PCOS patients showed a significant lower level of CD34+CD133+ cells (0.85% vs 1.34%; p < 0.009), CD34+CD184+ cells (0.019% vs 0.400%; p < 0.002) showing a reduction in circulatory EPC pool. PCOS patients also showed a higher level of CD146+ cell counts (1.000 vs 0.749%; p < 0.006) which is a marker for mature endothelial cells.

**Conclusions**
EPC levels are reduced in our PCOS patients, suggesting that repair and regeneration potential of endothelial functions are impaired. Insufficient circulating EPCs may be responsible for insulin resistance in the manifestation of PCOS. This finding also provides an additional potential basis for regarding PCOS as a risk factor to cardiovascular disease in women.
FC 12-2 Ovulation induction
Oral agents for ovulation induction: New horizons
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In this presentation we address some issues and new protocols of the oral agents used for ovulation induction. Early administration of CC, in the late luteal phase of the preceding cycle, in patients with PCOS will lead to greater follicular growth and increased endometrial thickness which might result in higher pregnancy rate. This novel protocol may improve the performance of CC as ovulation induction agent. Extended CC protocol (> 5 days therapy) starting day 2 of menses seems to offer economic, efficacy, and safety advantages and should be considered in some settings before moving to more expensive or sophisticated alternatives in cases of CC-resistant PCOS. N-Acetyl cysteine may be an effective adjuvant treatment to CC for inducing ovulation in polycystic ovary patients but not in cases of unexplained infertility. Letrozole does not show any advantage over clomiphene citrate as a first-line therapy for induction of ovulation in women with PCOS. Considering the cost of treatment, there is a remarkable difference in favor of CC. The same is true for anastrozole because CC is also cheaper in use- and more cost-effective. Anastrozole may be helpful in situations in which multiple pregnancies are not desirable or the risk of ovarian hyper-stimulation syndrome is high such as PCOS. When aromatase inhibitors are to be used, there are no significant differences expected in PR or miscarriage rate between anastrozole and letrozole employed for ovulation induction in women with CC-resistant PCOS. When letrozole is used for ovulation induction, it seems that the use of higher doses of offers no advantage in terms of pregnancy rates over the lower (2.5 mg) dose. Aromatase inhibitors and CC result in similar pregnancy outcomes and miscarriage rates. Our trial, in agreement with others, did not show any remarkable increase in the rate of congenital malformations.

FC 12-4 Polycystic ovaries
Metformin treatment before and during IVF or ICSI in PCOS women with BMI < 28 kg/m²: A prospective, multicenter, randomised, double-blind study
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Aims It is debated whether metformin co-treatment in IVF/ICSI significantly improve live birth, pregnancy or fertilisation rates [Cochrane review 2009].

Methods 8 clinics recruited a total of 149 PCOS (polycystic ovarian syndrome) women scheduled for IVF or ICSI treatment. The patients were randomised by the hospital pharmacy making identical capsules of metformin (74) and placebo (75). Codes were not broken until last ongoing pregnancy in the study was 32 weeks pregnant. All patients fulfilled the Rotterdam criterias, and all had BMI < 28 kg/m². All patients had general diet- and lifestyle counselling at screening, and were treated with metformin/placebo (1000 mg bid) 4–5 months until the day of positive/negative s-hCG (pregnancy test). Patients were downregulated with nafarelin at least 14 days before stimulation with recombinant FSH (fixed dose 112.5 IE). From cycle day 8 dose adjustments were allowed. Mean age was 29.5 years, and mean BMI 23.8 kg/m².

Results The primary endpoint in this study was clinical pregnancy rate defined as an intrauterine gestational sac by ultrasound week 7. According to intention to treat analysis the clinical pregnancy rate was 0.50 in metformin group vs. 0.33 in placebo group (p = 0.04). Live birth rates were 0.49 vs. 0.32 (p = 0.04). All babies born were singletons.

Prior to IVF 14 patients dropped out, and during pre-treatment 15 spontaneous pregnancies occurred in metformin group vs. 8 in placebo group (p = 0.10).

In per protocol analysis among patients starting gonadotrophin stimulation respectively 56 in metformin group vs 56 in placebo group; the biochemical pregnancy rate was identical 0.43 vs 0.43. There were a difference (although not significant) in clinical pregnancy rate (0.39 vs 0.30), and in live birth rate (0.38 vs 0.29).

There were no significant differences in the total number of oocytes (11.6 vs 13.2), fertilisation rate (0.53 vs 0.54), number of cleaved embryos day 2 (6.0 vs 6.6), number of transferred embryos (1.2 vs 1.1), number of frozen embryos (2.5 vs 2.7), and number of good quality embryos (3.5 vs 3.6). Total dose of gonadotrophin (1553 IE vs 1531 IE), and number of stimulation days (12.4 vs 12.1) were also similar.

Conclusions In this prospective randomised, double-blind study metformin treatment before and during IVF or ICSI increase live birth rates in PCOS women with BMI < 28 kg/m². The number of spontaneous pregnancies during pre-treatment contributes notably to this result.

FC 12-5 Ovulation induction
Clomiphene citrate versus Letrozole combined with gonadotropins in super-ovulation and IUI cycles – a comparative study
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Objective Letrozole is gaining popularity as a first line ovulation inducing agent with proposed advantages of lesser antiestrogenic side effects like thicker endometrium and mono follicular ovulation. To compare and evaluate the response to ovulation induction using clomiphene and letrozole combined with gonadotropin in intra-uterine insemination (IUI) cycles on the clinical, hormonal, ultrasound parameters and the adverse effects.

Design A prospective cohort study.
**Setting** Gynecology outpatient department in a tertiary institute in New Delhi, India.

**Materials and Methods** This study was done from June 2009 to February 2010 in infertile patients requiring IUI. The patients were alternately chosen into 2 groups: Group 1 clomiphene (n = 28) and Group 2 letrozole (n = 30). The patients received clomiphene 100 mg/day or letrozole 2.5 mg/day for 5 days from day 2–3 of cycle and in addition received human menopausal gonadotropin (hMG) 75 IU/day in a stepwise protocol till at least one mature follicle, followed by ovulation with hCG 10,000 IU. A single IUI was done 36 hours after hCG injection. The response to ovulation induction was compared in the 2 groups.

**Results** The 2 groups were comparable with respect to age, factors causing infertility and duration of infertility. Average dose of gonadotropins required (1: 590 IU; 2: 403 IU); endometrial thickness on the day of hCG (1: 7.82 mm; 2: 8.05 mm), number of follicles formed (1: 1.35; 2: 1.37) were similar in both groups. Estradiol levels on the day of hCG was significantly less in Group 2 (21 ± 41 vs 263 ± 74). Average pregnancy rates in the 2 groups were comparable (1: 11.1%; 2: 13.3%).

**Conclusions** Letrozole along with gonadotropins in superovulation and IUI cycles has similar ovulation and pregnancy rates with better endometrial thickness and more physiological estradiol levels compared to clomiphene with gonadotropins. Advantages with use of letrozole as an ovulation inducing agent do not emerge from these findings. Their use may be restricted to being a second agent do not emerge from these findings.

The study did not receive any funding from anywhere.

**FC 12-6 Ovulation induction**

Role of N-acetyl-cysteine for ovulation induction in clomiphene citrate-resistant PCOS women

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**Objective** To study the effect of N-acetyl cysteine as an adjuvant to clomiphene citrate on ovulation and conception rates in clomiphene resistant patients.

**Design** Placebo-controlled, randomized trial.

**Setting** Outpatient department of Obstetrics and Gynecology.

**Aims/Patients** 60 women diagnosed with CC-resistant PCOS, aged 18–35 years undergoing therapy for infertility were included.

**Intervention** The patients were divided randomly into 2 equal groups of 30 patients each. Those in group A were assigned randomly to receive either NAC 1.8 g/d (group A1) or placebo (group A2) 6 weeks prior to ovulation induction with CC 100 mg/d for 5 days starting from day 3 of cycle. Those in group B were assigned randomly to receive either NAC 1.8 g/d (group B1) or placebo (group B2) with CC 100 mg/d for 5 days starting at day 3 of the cycle.

**Main Outcome Measure** Ovulation rate and pregnancy rate (PR).

**Result** In group A pretreatment with NAC significantly increased both ovulation rate and PR in women with CC-resistant PCOS (73.3% vs 13.3%, 26.7% vs 0%, respectively). There was significant improvement in metabolic profile. NAC showed a significant decrease in LH levels and a significant improvement in LH/FSH ratio. A decreasing trend was seen in testosterone and estradiol levels but it was not statistically significant. No cases of ovarian hyperstimulation syndrome (OHSS) were reported in the NAC group; one case of miscarriage (25%) was reported.

**Conclusions** NAC as an adjuvant to CC was more effective than placebo for CC-resistant patients with PCOS. It is safe and well tolerated.

**FC 12-7 Polycystic ovaries**

Elevated level of serum anti-Müllerian hormone in polycystic ovary syndrome and it’s correlation with LH as well as free androgen index

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**Background** Polycystic ovary syndrome (PCOS) was characterized by disrupted folliculogenesis and arrested follicular maturation. The recent findings have shown that disruption in folliculogenesis and follicular maturation in PCOS was because of the high level of anti-Müllerian Hormone (AMH). The objective of this study is to describe the role of AMH as etiology of PCOS by comparing serum AMH level in PCOS group and control group. This study was also aimed to describe whether serum AMH level had correlation with LH, LH to FSH ratio, and Free Androgen Index (FAI).

**Materials and Methods** This study was a case-control study conducted in Cipto Mangunkusumo General Hospital, Jakarta. We analyzed 50 infertile PCOS subjects and matched them with 50 infertile non-PCOS subjects as control group. The diagnosis of PCOS was based on Rotterdam criteria. We measured basal serum FSH, LH, prolactin, testosterone, sex hormone binding globulin (SHBG), FAI, and correlated them with serum AMH level.

**Results** Serum AMH level was found to be significantly elevated in PCOS group compared to non-PCOS group (9.43 ± 5.40 ng/ml vs 3.08 ± 3.54 ng/ml; p < 0.0001). LH level was also higher in PCOS group (10.06 ± 8.44 mIU/ml vs 4.60 ± 2.32 mIU/ml; p < 0.0001). This might explain the role of AMH as the cause of abnormal follicular development and arrested follicular maturation in PCOS patients. There was significantly positive correlation between serum AMH level and LH level (p < 0.0001; r = 0.425), as well as between serum AMH level and LH to FSH ratio (p < 0.0001; r = 0.560). We also found weak but positive correlation between serum AMH level and FAI (p = 0.030; r = 0.384) in PCOS patients.

**Conclusions** Serum AMH level, LH level, and LH to FSH ratio were significantly higher in PCOS group compared to non-PCOS group. There was significant correlation between serum AMH level and LH level, between serum AMH level and LH to FSH ratio, as well as between serum AMH level and FAI.

**FC 12-8 Ovulation induction**

Novel treatment for Luteinized Unruptured Follicle (LUF) using Granulocyte Colony-Stimulating Factor (G-CSF)

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**Objectives** Many cytokines are involved in ovulation. We have previously reported that granulocyte-colony-stimulating factor (G-CSF) and its receptor are found in the follicular wall just prior to ovulation and are closely involved in the mechanism of follicle rupture. Luteinized Unruptured Follicle (LUF) is observed in patients with unexplained infertility at the rate of more than 10%. The cause of such LUF might be a disorder of the mechanism of follicle rupture. For this reason, we have conducted a clinical trial for LUF patients using G-CSF and obtained the anticipated results.

**Materials and Methods** Subjects were 65 consenting infertile women who showed a LUF at previous Clomiphene-hCG cycle by transvaginal ultrasonography and basal body temperature. At the consecutive cycle, the same dose of Clomiphene-hCG and 100 µg of G-CSF was administered at 24–48 hours before hCG administration. The rates of LUF before and at G-CSF cycle were statistically compared.

**Results** The total numbers of the G-CSF cycles were 73. Age of the patients was 32.9 ± 3.8 years (Mean ± S.D.) and the average numbers of treated cycles up to G-CSF administration was 7.4 ± 3.5 cycles. Of the total of 148 cycles before G-CSF administration, LUF was observed in 76 cycles (Ovulation rate: 48.6%). By the administration of G-CSF, ovulation was confirmed in 64 of 73 cycles (87.7%), which is significantly higher than the cycles without G-CSF (p = 0.00000002). Four patients participated in this study became pregnant at G-CSF cycle.
Conclusions

The similarity between ovulation and inflammation has been confirmed during the last 30 years. Nevertheless, little attention was paid to the granulocytes in the ovulation mechanism, which are the most mobilized leukocytes during inflammation. G-CSF administration during CC-hCG treatments was very effective to prevent LUF. Since there are no established and definite treatments for LUF present, the treatment with G-CSF must be tried for LUF patients. Of course, further trials are necessary. However, the above results indicate the possibility of G-CSF as a supportive drug in ovulation induction.

FC 13-1 Polycystic ovaries
Assessing correlation between ovarian & stromal volumes and fasting & postprandial insulin levels in PCOS patients

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Background

Polycystic ovarian syndrome is a metabolic disease. These females have hormonal changes, some times subtle but have significant impact on long term health. Patients with polycystic ovarian syndrome are believed to have large ovaries due to increased stroma and large number of antral follicles on ultrasound. They also have derangement in LH and testosterone levels, high insulin resistance and bad lipid profiles. As insulin resistance is thought to be associated with endocrinological and ultrasound signs, correlation was expected interevene.

Aim

To assess if ovarian and stromal volumes in polycystic ovarian syndrome patients have any correlation with fasting and postprandial insulin levels.

Design and Settings

A prospective randomized study of 50 polycystic ovarian disease patients was done over a period of 6 months with clinical examination, baseline ultrasound scan with 2D, colour and spectral Doppler and 3D ultrasound and fasting and postprandial insulin levels.

Method

All patients with oligoovulation and/or hirsutism and Acne, were scanned by 2D and colour Doppler ultrasound on day 3 of the cycle. Patients with polycystic ovarian syndrome (PCOS) according to Adem Balen’s definition were selected for the study and were further assessed by 3D and 3D power Doppler (3DPD) ultrasound (US) for calculation of ovarian and stromal volumes and fasting and postprandial insulin levels on the same day. For all patients ovarian volume and stromal volume were calculated by VOCAL.

Results

Positive correlation was seen between ovarian and stromal volumes and fasting and postprandial insulin levels. With Pearson correlation significance level of 0.01 (2 tailed) the correlation for ovarian volume to fasting insulin is 0.588, for ovarian volume to post prandial insulin is 0.523, for stromal volume to fasting insulin is 0.580 and for stromal volume to post prandial insulin is 0.523.

Conclusions

In PCOS patients a strong correlation is found between ovarian and stromal volume and fasting and post prandial insulin levels.

FC 13-2 Dyslipidemia in women with Polycystic Ovary Syndrome (PCOS)

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Introduction

The long term health consequences of PCOS include an increased prevalence of diabetes, dyslipidemia and cardiovascular disease with a possible impact on morbidity and mortality. This study was undertaken to describe the various lipid disorders in a cohort of patients with PCOS attending a referral hospital clinic in South Africa. We attempted to describe dyslipidemias encountered and to assess the interrelation-ship of the lipid profile with anthropometric and endocrine factors.

Methods

All women diagnosed with PCOS had a clinical, endocrine and metabolic assessment performed on presentation. The lipid profile included triglycerides (TG), cholesterol, high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc). In addition LDL fractionation was performed to identify particle size, as the small dense particles carry a greater cardiovascular risk.

Results

A total of 1 059 women were included in the study and 1 038 lipid profiles were available for analysis. The age range was 12–46 years (mean ± SD: 26.6 ± 5.9) and body mass index (BMI) ranged from 15.4 to 62 kg/m² (mean ± SD: 32 ± 18.3). The clinical presentation included menstrual dysfunction (82%), hirsutism (60%) and infertility (73%). A total of 754 women had at least one lipid abnormality and 77 had abnormalities carrying significant cardiovascular risk. These included severe hypercholesterolemia (n = 37), hypertriglyceridemia of > 5 mmol/L (n = 9), low LDLc (< 1.0 mmol/L n = 8), low HDLc (< 0.7 mmol/L n = 11), high HDLc (n = 6) and dysbetalipoproteinemia (no normal LDLc; n = 6). LDLc fractionation demonstrated that the smaller particles were negatively related to age, BMI, waist hip ratio, androgen status and glucose/insulin ratio (all p < 0.0001).

Conclusions

Women with PCOS in our population have a high prevalence of dys-lipidemia. These data highlight the importance of early metabolic assessment of all women with PCOS. Long term follow-up and appropriate intervention are essential. Weight control and treatment of hyperandrogenism and insulin resistance may improve the long term risks for these patients.

FC 13-3 Polycystic ovaries
In-vitro maturation of oocytes – Can a classification of cumulus oocyte complexes (COCs) of oocytes obtained from patients with polycystic ovarian syndrome predict maturation outcome?

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Objective

Significant progress has been made in in-vitro maturation (IVM) of immature oocytes. This method can eliminate the risk of ovarian hyperstimulation syndrome (OHSS) in PCOS patients.

Aim

To summarize our experience and outcomes of IVM treatment in PCOS patients and to correlate maturation of in-vitro matured oocytes with cumulus-oocyte complexes (COCs) classification.

Design

Prospective study.

Materials and Methods

27 PCOS patients, aged 21–35 years, underwent 33 IVM cycles between 01.05.2008 and 30.04.2009. Patients were priming with fSH (150 IU) for 3 days in early follicular phase. When leading follicle was 8–12 mm, hCG (375 µg) was administered 38 hours prior ovum pick up. Follicles were aspirated by a 19G (Cook, Australia) lumen needle. Collected COCs were classified in 5 groups:

1. expanded cumulus
2. full cumulus
3. partial cumulus
4. atretic
5. nude

Mature oocytes were injected after 6 hours of incubation in IVM medium (Medi-Cult). Others were further incubated for 30 hours in IVM medium. Maturation and fertilization rates were compared between the groups. Statistical analysis was performed using Fisher exact test.

Results

Total of 442 oocytes were collected (13.4 ± 5.7 per cycle) and classified:

1. 139
2. 185
3. 58
4. 45
5. 15 oocytes, respectively

Total maturation and fertilization rates were: 64.9% and 44.2% respectively. Maturation rate according to COCs classification was:

1. 79.9%
2. 65.9%
3. 63.8%
4. 46.7%
5. 60%, respectively
Significantly higher maturation rate was detected in group 1 vs 2 (p = 0.0061), vs 3 (p = 0.029), vs 4 (p < 0.001) respectively, as well as vs 5 (p = 0.025). Total cleavage rate was 94% and 56% of embryos were of good quality. 91 embryos were transferred in 31 cycles, 7 pregnancies issued (22.6% per ET).

Conclusions Favourable results were obtained using IVM protocol in PCOS patients however still lower as compared to IVF cycles. Oocytes originated from expanded cumulus may have a higher potential of in-vitro maturation, since these oocytes started already in-vitro maturation process triggered by hCG administration. Therefore it can be assumed that embryos developing from these oocytes may have a better implantation capacity. Moreover, significantly higher in-vitro maturation rate of full cumulus oocytes as compared to partial cumulus oocytes, can indicate that existence of intact cumulus all around the oocytes may have a crucial role in in-vitro maturation process.

FC 13-4 Polycystic ovaries
Benign breast disease and estrogen-progesterone therapy by infertility and polycystic ovary syndrome

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Objective Polycystic ovary syndrome (PCOS) is a known entity correlated to metabolic syndrome and chronic anovulation. Our study aims to compare PCOS patients with infertile women undergoing previous in vitro fertilization (IVF) procedures and healthy women among patients affected by benign breast disease (BBD). The aim of this study is to evaluate the effect of PCOS, infertility and hormone intake on BBD.

Materials and Methods We collected data about all women with BBD in their fertile age who applied the Senology Outpatients during 2008, paying attention to their eventual gynecologic comorbidities. We analyzed data by R (version 2.10.0) considering significant p < 0.05. Also multivariate analysis was performed.

Results Among 105 women with BBD, 8 resulted affected by PCOS, 63 women have been treated with EP therapy, and 4 women have been treated with IVF. Patients treated with IVF had an higher prevalence of fibrocystic pathology (50%) than PCOS (35%) (p = 0.024), and on the other hand, in IVF patients and PCOS there was a lower prevalence of fibroadenoma (25%, 33% vs 61%). Pregnancy achievement was significantly less prevalent in PCOS and IVF patients (13%, 25% vs 48%); who had also a higher prevalence of hormone use (100%, 73% vs 56%). PCOS, IVF procedures and EP intake resulted to be more predictive for fibrocystic pathology then for fibroadenoma, presenting a ROC curve with an AUC of 69%.

Conclusions Higher hormone milieu seems to be correlated and predictive of fibrocystic pathology.

FC 13-5 Polycystic ovaries
Features of passing PCOS, depending on the polymorphisms of genes IRS2, AR, and PPARG

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PCOS actively studied for many decades, this syndrome is still not fully understood. Numerous researchers noted that the group of patients is heterogeneous in clinical manifestations from the random finds enlarged ovaries according to ultrasound to express hirsutism, obesity and insulin resistance. It is presently considered that development of syndrome is the result of interaction of many groups of genes between itself and with the factors of external environment, so disease is subject to the principles of multifactorial inheritance.

Numerous studies have confirmed the main role of insulin in the pathogenesis of metabolic disturbances in polycystic ovary syndrome. Focus of attention is displaced from the ligand to the receptor mechanism and ways of transmission inside the cell, in particular the emerging work on the integration of the insulin receptor substrate-2, androgen receptor (AR) and peroxisomes proliferators-activated receptor-g (PPARG) in the pathogenesis of PCOS. Were identified single-nucleotide polymorphisms (SNPs) associated with different clinical variants of manifestations PCOS. However, the literature contains no data on the role of these polymorphisms in Russia’s population.

The purpose of this research was to study the frequency of occurrence of polymorphisms of genes IRS2: G1057D (rs1805097), PPARG: P12A (rs1801282) and the number of CAG-repeats in the gene AR (rs902610) and their relation to the various versions of the manifestations PCOS.

Materials and Methods The study included 150 women, of whom 100 women with PCOS, they recruited the following criteria for the diagnosis of PCOS (The 2003 Rotterdam consensus workshop, 2004) and 50 somatically healthy women with ovulatory menstrual cycles. All women evaluated the clinical and anamnetic parameters (insulin resistance, morphological parameters, hormonal profile). Molecular-genetic analysis was made using fluorescent hybridization probes and melting curve analysis to detect IRS2 G1057D and PPARG P12A polymorphisms. CAG repeat length in the AR gene was estimated using a PCR-based assay. The amplified products were separated on a denaturing polyacrylamide gel.

FC 13-6 Polycystic ovaries
Association of the T45G and G276T polymorphisms in the adiponectin gene with PCOS: A meta-analysis

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Aims Adiponectin is the most abundant adipocytokine and may play a role in the pathogenesis of polycystic ovary syndrome (PCOS). There are conflicting data concerning the association between PCOS and 2 polymorphisms of the adiponectin gene, T45G and G276T. To clarify the association of these 2 polymorphisms with PCOS, a meta-analysis were performed in this study.

Methods Literature search was conducted through PubMed, EMBASE and references from relevant studies. Pooled odds ratios (OR) were estimated using fixed effects (FE) models in codominant, recessive and dominant models. Sensitive analysis was performed by excluding invalid studies.

Results We identified 7 published articles which reported the T45G polymorphism in PCOS, and in which 5 ones associated with the G276T polymorphism in PCOS. We found significant association of adiponectin T45G polymorphism with PCOS in any of codominant (FEM: OR = 1.34, 95%-CI: 1.09–1.65), recessive (FEM: OR = 2.02, 95%-CI: 1.12–3.64) or dominant models (FEM: OR = 1.32, 95%-CI: 1.03–1.68). For adiponectin G276T polymorphism, the OR and 95%CI are 0.81 (0.08, 0.98), 0.74 (0.51, 1.09) and 0.76 (0.61, 0.99) in codominant, dominant and recessive models.

Conclusions This study provides evidence for an association of the adiponectin gene with PCOS that needs to be confirmed by further studies.

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FC 13-7 Polycystic ovaries
The effect of Zone regimen on BMI rate in polycystic ovarian syndrome women

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Introduction BMI or body mass index is a major criterion for detection of obesity and overweight. Therefore it shows that your weight is sufficient for your height or not. In polycystic ovarian syndrome women obesity and increasing of BMI is a major problem in many of them (more than 50%). It can aggravate symptoms and sign of disease and lead
to sever unhealthy condition like cancers in non affected and affected women by PCOs. Many of research showed that weight loss has positives effect on health women progressing. Therefore we try to use low carbo-
hydrate especially zone regimen to check this thesis that low glucose intake in non re-
stricted regimen can be more effective on quick BMI decreasing.

Materials and Methods This is an inter-
ventional study and we used simple sampling 
method to collect data. We designed our 
study with 24 PCOs women who were oligo-
menorhea, overweight, obese and did not 
have another hormonal disorder, then we ad-
vised zone diet with 1500 kcal/day (non re-
stricted regimen) and aerobics exercise (twice a week). After each week, samples 
completed a 24 hour nutrition recall and after 
each month weight and height was recorded 
and BMI been calculated. Data was analyzed 
using the SPSS software version 14.

Results 
Average quality of weight loss in 
first month was (1/45 kg), in second month 
was (3/221) and third month was (5/046). 
The Wilcoxon test showed significant differ-
cence in weight loss at the third month com-
pared to the beginning of study (p = 0/004). 
Mean of BMI at first month was 2/32 kg/m2, at 
second month was 0/845, and third month 
was 0/001. Wilcoxon test showed signi-
ficant changes of BMI in third month com-
pared start of study (p = 0/001).

Conclusions Zone diet as a low carbo-
hydrate regimen can be advised by physician to 
all overweight people like poly cystic ova-
rian women that they are interested to use a 
non restricted and daily regimen. By this 
regimen BMI can be reduced in short time 
and without any major force on caloric in-
take. We hope that by using of this regimen 
weight overcomplications be recovered and 
we advise it for all obese people.

FC 13-8 Polycystic ovaries
PCOD is a physiological adaptive 
reaction to prevent multiple preg-
nancies: Studies on 10 large fami-
lies and 44 single cases with 
PCOD with a mechanism guided 
approach
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2Mehr Medical Group, Gynecology, Tehran, Islamic 
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Objectives Evaluation whether PCO can be 
a natural mechanism against the risk of 
multiple pregnancies (MP); developing a 
mathematical model on mediators control-
lng menstrual cycles.

Materials and Methods 10 large fami-
lies and 44 PCOD cases were studied in 3 
generations.

Results Based on the Hardy Weinberg law 
of population genetics, it is improbable that 
PCO with such a high frequency comprises a 
disease. Different hormonal controlling loops 
of reproduction can be simplified into a for-
mula which encompasses a direct mediator 
from the ovaries affecting hypothalamus. 
This formula also explains psychological 
 phenomena affecting fertility.

Conclusions 1. MP s in pre-midwifery era were uniformly 
fatal for mother, fetus or both. A gene 
with a frequency of 5–14% must harbor 
survival advantage for the patient or her 
offspring. Prevention of MP is the only 
explanation.
2. Unlike bone narrow ovaries do not have a 
chastic mechanism and rely solely on 
suppression of nearby follicles by the 
dominant follicle.
3. Hypothalamus senses the risk of MP and 
adjusts pulsation of GnRH in the consecu-
tive cycle by prolonging the low and post-
poning the high frequency pulsation. 
Continuation of MP risk for four consecu-
tive cycles causes complete cessation of 
GnRH pulsation, stabilization of FSH and 
LH, anovulation and development of 
PCO pattern.
4. Infertility in PCO is relative and due to 
high risk of MP. A yet unknown ovarian 
mediator that informs the hypothalamus is 
responsible for reduced GnRH pulsation 
and blocking it will cure PCO in all pa-
tients compared to Metformin, Clomi-
ophene or aromatase inhibitors (20–64%).

FC 14 Infertility

FC 14-1 Oocyte
Gene expression analysis of 
single oocytes from young and 
aged mice
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2DNA Chip Research Incorporation, Yokohama, Japan

Introduction Gene expression analysis of 
single oocytes is a useful tool for research in 
assisted reproduction technologies. How-


amplification procedure was also assessed 
by quantitative PCR with a TaqMan probe 
using 4 abundantly expressed genes.

2. Morphologically normal oocytes from 6 
weeks (young) and 44 weeks (aged) old mice 
were analyzed.

Results 
1. Duplicated amplification reactions and 
array analysis with the same RNA sample 
(500 pg) yielded signal correlations with 
r = 0.99. The correlations of the results 
between the RNA sample extracted 1 and 
500 oocytes was 0.83. Linear correlations 
in the expression levels of the 4 genes be-
fore and after amplification were noted.
2. Based on cluster analysis of the micro-
array data, nine oocytes from the 3 young 
mice showed highly uniform global gene 
expression profiles. On the other hand, 6 
oocytes from the aged mice showed sig-
ificantly heterogeneous gene expression 
profiles. The genes and pathways which 
were significantly downregulated in the 
oocytes from the aged mice as compared 
with those from the young included those 
related to cell-cycle regulation and 
spindle-assembly.

Conclusions Reproducible array perform-
ance was achieved with total RNA extracted 
from single oocytes using this system. Utiliz-
ing this protocol, oocytes from aged mice 
showed different expression profiles as com-
pared to those from young mice, and the 
pathway analysis performed on these studies 
suggested that the high probability of chro-
mosome aneuploidy among the oocytes from 
age mice might be explained by the change 
of expression among the molecules related to 
spindle configuration, although validation 
study using quantitative PCR should be nec-
sary.

FC 14-2 Infertility diagnosis
A new evaluation score using 
salpingoscopy that reflects fallo-
opian tube function in infertility 

women
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cal School, Tokyo, Japan

Purpose Although laparoscopy remains to 
be the gold standard for the evaluation of fal-
lopian tubes, the process cannot be used to 
directly observe the inner cavity of the fal-
lopian tube. Salpingoscopy, however, can be 
used to directly assess the mucosa of the in-
fundibulum and ampulla. Therefore, we per-
formed salpingoscopy concomitantly to the 
performance of laparoscopy to glean more 
information about the fallopian tube. In this 
study, we tried to build an evaluation scoring 
system using the results of salpingoscopy, 
and to evaluate the relationship between this 
scoring system and the outcome of preg-
nancy.
Materials and Methods From April 2008 through June 2009, we recruited a total of 104 women for this study who had been diagnosed with unexplained infertility and who underwent both laparoscopy and salpingoscopy at our clinic. We observed the right and left tubal lumen paying particular attention to the following 6 findings:

1. Adhesions
2. Loss of mucosal folds
3. Rounded edges of mucosal folds
4. Debris
5. Foreign bodies, and
6. Abnormal vessels by salpingoscopy.

After salpingoscopy, we calculated the F score which was our original score expressing the sum of the abnormal findings. One abnormal finding was given a 1-point of F score, the maximum was 12 points. We analyzed the relationship between the F scores and various clinical findings or pregnancy rates.

Results The average age of the patients was 34.2. The average F score was 0.9 ± 0.1 (mean ± S.E.M), and the range was from 0 to 7. Slightly more than half (59.7%) of the patients showed an F score of 0, and the percentages of patients who showed 1, 2, 3 and 4 or more points were 19.2%, 11.5%, 4.5%, and 4.5%, respectively. The average F score for patients showing positive chlamydial antibodies was 1.7 ± 0.4, and which was significantly higher than that of patients who showed negative (0.6 ± 0.1; p = 0.0003). After evaluation, 23 patients achieved pregnancy within a year. The pregnancy rates of the group whose F score was 0 or 1 point were 27.4% and 20.0%, respectively, and these were significantly higher than those of the group whose scores were 2 or 3 or more points at 8.3% and 10.0%, respectively (p < 0.05).

Conclusion Homocysteine has an inverse effect on sperm count and membrane integrity as shown by the elevation of malondialdehyde concentration which reveals the lipid peroxidation in sperm membrane.

FC 14-4 Infertility diagnosis
Recently characterised recombinant antigens improve detection of antibodies against Chlamydia trachomatis in patients with tubal occlusion

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Introduction Infections with Chlamydia trachomatis represent a significant risk of infertility due to tubal pathology. Conventional serological testing for Chlamydia antibodies has been hampered by low sensitivity and specificity. We tested infertile women with tubal occlusion, patients with recurrent pregnancy loss (RPL), healthy pregnant controls and blood donors (BD) for antibodies against C. trachomatis using line immunoassay/ELISA respectively. Only 1 of 27 IgA-reactive patients with tubal occlusion showed reactivity with HSP60 but 8/19/20 with MOMP/OMP2/TARP/CFAP.

Conclusions Applying newly developed assays against C. trachomatis, we detected a significantly increased prevalence of IgG and IgA antibodies against C. trachomatis in patients with tubal occlusion compared to healthy pregnant women, RPL-patients and blood donors. These findings point to a past (IgG) and/or silent (IgA) C. trachomatis infection. These newly developed assays, that for the first time utilize the recombinant antigens (CIFAP, TARP) may help to identify a high percentage of patients with sterility due to tubal occlusion. These new assays appear to facilitate identification of tubal factor infertility.

FC 14-5 Oocyte
Formation of two-celled oocytes and chimeric embryos by GV oocytes

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Objective We have previously reported that blocking spindle rotation in mouse MI oocytes prevented PB extrusion and resulted in the formation of two cells of similar size within the zona pellucida. Our aim was to determine whether in such oocytes each cell could be fertilized by separate sperm and whether this would lead to the formation of chimeric embryos.

Methods Informed consent was obtained from patients who provided GV oocytes following ovarian stimulation for ICSI. Time-lapse recordings were done on the human GV oocytes cultured in a CO2 chamber equipped with a DIC inverted microscope. Gentle compression of MI oocytes blocked the rotation of the meiotic spindle and induced the formation of two cells of similar size in each oocyte. Mouse oocytes were used for studies on the fertilizability of such 2-celled oocytes. This was done by in vitro...

Whereas, the membrane integrity was evaluated according to hyposmotic swelling test (HOS-Test). Besides, the homocysteine concentration was measured by high performance liquid chromatography (HPLC) techniques and the malondialdehyde level was measured using chemical method. (Thiobarbituric acid; TBA).

Results The mean values of spermatozoa concentration, motility and membrane integrity in all investigated samples were (58.20 ± 54.40 mill/ml; 17.34 ± 23.98% and 76.51 ± 15.02%). Homocysteine concentration was 20.2 ± 4.9µmol/L, while malondialdehyde level was 3.7 ± 1.2 µmol/L.

A positive correlation was found between homocysteine and malondialdehyde concentration in seminal plasma (r = 0.287; p = 0.147). Whereas a negative correlation was observed between homocysteine and sperm count (r = 0.401; p = 0.08) as well as with sperm membrane integrity (r = -0.209; p = 0.235).

Conclusion Homocysteine has an inverse effect on sperm count and membrane integrity as shown by the elevation of malondialdehyde concentration which reveals the lipid peroxidation in sperm membrane.
insemination of the 2-celled oocytes after partial disruption of their zona with laser. Fertilization and embryonic development were observed and photographed at frequent intervals.

**Results**

We have filmed the sequence that established two intra-zonal cells in both human and mouse oocytes. When such 2-celled MII mouse oocytes were inseminated, in the presence of a small opening in their zona pellucida, a typical second polar body was exuded from each cell and male and female pronuclei were formed in each fertilized cell. Subsequently, each cell cleaved to form twin embryos within the same zona pellucida. The twin embryos amalgamated at the morula stage to form a single compacted morula which progressed to hatched blastocysts that had a single inner cell mass. We need to determine whether these contained double maternal and double paternal DNAs.

**Conclusions**

Fertilization and development of two separate cells within the same zona pellucida may be one mechanism that leads to the formation of hermaphrodites when one cell is fertilized by an X and the other by a Y sperm. To evaluate this proposal we have filmed the sequence that led to the formation of the MII oocytes. These embryos were transferred 2 days after the fertilization at a 2–3 cells stage, containing 10–20% fragmentation. A singular pregnancy with foetal hearth beat was detected. During prenatal care, the foetus was diagnosed healthy aside from a double-sinused left kidney. She gave birth to a newborn with caesarean section at 39th week of pregnancy.

**Conclusions**

Our case report demonstrates that serious oocyte abnormalities can be a recurrent phenomenon in the same patient. Cytoplasmic vacuoles can reduce the fertilization ability of oocytes. However, presence of large vacule(s) (50–60 µm) do not completely block the fertilization process. Oocytes containing large vacule can be normally fertilized and results in a normal embryo development and a viable offspring. Tight prenatal care and follow-up has to be carried out after transferring embryos developed from abnormal oocytes.

**FC 14-6 Oocyte Human oocyte containing large cytoplasmic vacuole can result in pregnancy and viable offspring**


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**Introduction**

Morphological characteristics of human oocytes may indicate developmental potential of the subsequent embryo. However, the correlation between these abnormal oocyte phenotypes and fertilization, embryo development is still unclear. In this case report we demonstrate that serious oocyte dismorphism can result in a normal fertilization and embryo development.

**Materials and Methods**

Following 2 years of infertility a 36 years old women reported to our clinic. Baseline hormonal assessment showed normal values. The anatomy of uterus was normal, the left tube was blocked. The male partner had severe oligo-, asthenoo-, teratozoospermia with only few progressive motile sperm. The couple underwent 5 IVF-ICSI treatments in our clinic between November 2004 and March 2005.

**Results**

42 oocytes were collected during the 5 IVF treatment. 76.2% of the oocytes (32) were mature (MII), but 26 MII oocytes (81.3%) contained at least one large cytoplasmic vacuole. The size of the vacuoles was between 29.2 µm and 61.0 µm, the average diameter of vacuoles was 52.2 µm. Zona pellucida of the MII oocytes were unusually thin. The mean thickness of the zona was 9.8 µm. Only 7 oocytes showed normal (2PN) fertilization out of the 42 MII oocytes injected. Thus, the fertilization rate was very low (16.7%) and 5 zygotes developed from oocytes contained large vacuoles. The first 4 IVF-ET treatments did not result pregnancy. In the fifth IVF treatment all oocytes contained at least one large vacuole. Two oocytes showed normal (2PN) fertilization and an other oocyte showed 1PN fertilization. These embryos were transferred 2 days after the fertilization at a 2–3 cells stage, containing 10–20% fragmentation. A singular pregnancy with foetal hearth beat was detected. During prenatal care, the foetus was diagnosed healthy aside from a double-sinused left kidney. She gave birth to a newborn with caesarean section at 39th week of pregnancy.

**Conclusions**

We have filmed the sequence that led to the formation of the MII oocytes. These embryos were transferred 2 days after the fertilization at a 2–3 cells stage, containing 10–20% fragmentation. A singular pregnancy with foetal hearth beat was detected. During prenatal care, the foetus was diagnosed healthy aside from a double-sinused left kidney. She gave birth to a newborn with caesarean section at 39th week of pregnancy.

**Conclusions**

Our case report demonstrates that serious oocyte abnormalities can be a recurrent phenomenon in the same patient. Cytoplasmic vacuoles can reduce the fertilization ability of oocytes. However, presence of large vacule(s) (50–60 µm) do not completely block the fertilization process. Oocytes containing large vacule can be normally fertilized and results in a normal embryo development and a viable offspring. Tight prenatal care and follow-up has to be carried out after transferring embryos developed from abnormal oocytes.

**FC 14-7 Infertility diagnosis Clinical study in men with infertility**

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**Objectives**

The aims of this case-control study were to identify the prevalence of the clinical & laboratory risk factors for male infertility among infertile men in Hilla city. Also to identify the seminal fluid patterns in these subfertile men as well as in fertile controls and to demonstrate the types of serum hormones (Follicle Stimulating Hormone (FSH), Leutinizing(LH),Testosterone and Prolactin) abnormalities in the study groups.

**Materials and Methods**

This study included 81 Iraqi subfertile men and 30 fertile men who fulfilled the selection criteria. Relevant history and physical examination were obtained. Besides, seminal fluid analysis (SFA) was performed according to WHO method. The patients group was subdivided by SFA results into aozoospermic, oligo-asthenozoospermic and oligozoospermic subgroups. Serum levels of the hormones (Testosterone, FSH, LH and Prolactin) were measured for patients and controls using ELIZA immunoassays. Seminal fluid analysis parameters mean levels and serum hormone levels were compared for the groups using Analysis of Variance test.

**Results**

Some clinical risk factors for male infertility appeared to be more prevalent in infertile men than in controls. These include history of wife’s abortion, decreased libido, low socio-economic status, low use of contraceptives, family history of infertility, chronic diseases, varicocele, scrotal surgery, testicular biopsy, smoking, excessive heat exposure and atrophied testes. Subfertile men showed lower values for SFA parameters than did the controls. Patients with azoospermia showed the most remarkable hormonal abnormalities especially in the levels of serum FSH and Testosterone. There was significant differences in the serum sex hormones levels between the patients and controls groups and among the infertile men subgroups.

**Conclusion**

Iraqi infertile men have certain clinical, seminal and hormonal abnormalities which are different from fertile controls. Moreover, the differences are present between patients subgroups themselves and these abnormalities are multi-components. Patients with low sperm concentration and especially those with azoospermia are those that most likely will get benefit from hormonal assays. Serum FSH and Testosterone are the best 2 hormones for initial male infertility evaluation.

**FC 14-8 Infertility diagnosis 24 hours urinary Prolactin (24UP) is the best marker to differentiate normal from micro/macroadenoma: Prolactinoma is a natural family planning not a disease entity**

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**Objective**

To define the clinical significance of Prolactinoma and find more reliable laboratory markers we tried to use the 24 hours urinary Prolactin.

**Materials and Methods**

74 normal volunteers were compared with 12 hyperprolactinemia of unknown significance (HPUS) and 38 confirmed Prolactinoma for 24UP corrected for volume and Creatinin clearance. Hyperprolactinemia of unknown significance and confirmed Prolactinoma were defined as elevated blood Prolactin level with 0–1 and all three criteria ofamenorrhea, galactorrhea and headache respectively. Blood and urine samples were evaluated with highly sensitive ICT. Prolactinoma was evaluated with dynamic MRI of the sella turcica.

**Results**

The mean and range of 24UP (as such and corrected) were completely different among the three groups with 11–17, 14–38 and 26–49 microgram/d respectively. 24UP could differentiate macro from micro adenomas. This will reduce the need for MRI in most controls. Patients with azoospermia showed the most remarkable hormonal abnormalities especially in the levels of serum FSH and Testosterone. There was significant differences in the serum sex hormones levels between the patients and controls groups and among the infertile men subgroups.

**Conclusion**

Iraqi infertile men have certain clinical, seminal and hormonal abnormalities which are different from fertile controls. Moreover, the differences are present between patients subgroups themselves and these abnormalities are multi-components. Patients with low sperm concentration and especially those with azoospermia are those that most likely will get benefit from hormonal assays. Serum FSH and Testosterone are the best 2 hormones for initial male infertility evaluation.

**FC 14-9 Infertility diagnosis 24 hours urinary Prolactin (24UP) is the best marker to differentiate normal from micro/macroadenoma: Prolactinoma is a natural family planning not a disease entity**

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**Objective**

To define the clinical significance of Prolactinoma and find more reliable laboratory markers we tried to use the 24 hours urinary Prolactin.

**Materials and Methods**

74 normal volunteers were compared with 12 hyperprolactinemia of unknown significance (HPUS) and 38 confirmed Prolactinoma for 24UP corrected for volume and Creatinin clearance. Hyperprolactinemia of unknown significance and confirmed Prolactinoma were defined as elevated blood Prolactin level with 0–1 and all three criteria ofamenorrhea, galactorrhea and headache respectively. Blood and urine samples were evaluated with highly sensitive ICT. Prolactinoma was evaluated with dynamic MRI of the sella turcica.

**Results**

The mean and range of 24UP (as such and corrected) were completely different among the three groups with 11–17, 14–38 and 26–49 microgram/d respectively. 24UP could differentiate macro from micro adenomas. This will reduce the need for MRI by 90%.

**Conclusions**

1. 24UP is the best marker for differentiating spurious hyperprolactinemia from prolactinoma.
Metabolic disturbances were recognized during ovulation induction (Mg 19.1%, albumin 11.3%, protein 14.2%, alkaline phosphatase 14.5%) and was associated with elevated Cl of 7%.

Conclusions Restoration of Mg, retinol, carotinoid and protein levels may indirectly normalize electrolytes concentration (K, Na, CI and also alkaline phosphatase) resulting to the improvement of hepatocyte function, compensate metabolic dysfunction and increase the success rate of IVF by means of improvement in metabolic processes in myocytes and provide OHSS and preclampsia prevention.

Introduction Ovulation induction may cause degeneration of liver membrane resulting to the decrease in alkaline phosphatase level. Such alterations lead to accumulation of lysosomal enzymes in pathological tissues and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system) a low Mg level. Such alterations lead to accumulation of lysosomal enzymes in pathological tissues and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system). A low Mg level and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system). A low Mg level and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes. A low Mg level and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system). A low Mg level and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system). A low Mg level and low activity in plasma associated with more significant metabolic disturbances including electrolytic disbalance and result in the evident homeostasis changes (ovarian pathology in reproductive system).

Objectives To study metabolic disturbances and their role in IVF program.

Materials and Methods 47 women of reproductive age were enrolled in the study. Mean age was 29.8 ± 4.1 yrs with infertility period 4.8 ± 1.9 yrs. 21 women have past history of IVF failure. Patients underwent a full clinical assessment and laboratory examination before IVF including electrolytes, enzymes, magnesium, vitamin A and carotinoids in blood plasma.

Results Metabolic disturbances were registered in 71.3% patients before IVF: low level of proteins including albumin, retinol, carotinoids, electrolytes (K, Mg, Na), alkaline phosphatase associated with high level of serum Cl 69% of patients had isolated low level of antioxidants (retinol and carotinoids). Among women with associated pathology in reproductive system a low Mg level was demonstrated in 83.1% patients and reached 20% decrease. The level of albumin and Mg in women with concomitant pathology (previous history of surgery for ovarian benign tumor, thyroid dysfunction and adenomyosis) was significantly reduced (32.3 ± 1.2 g/l and 0.52 ± 0.9) that may indicate the decrease in metabolic activity and adaptation value of hepatocytes. Alkaline phosphatase level was borderline low 89.0 ± 2.6 mmol/l evidences of cholestasis. Negative tendency for these parameters was demonstrated during ovulation induction (Mg 19.1%, albumin 11.3%, protein 14.2%, alkaline phosphatase 14.5%) and was associated with elevated Cl of 7%.

Conclusions Restoration of Mg, retinol, carotinoid and protein levels may indirectly normalize electrolytes concentration (K, Na, Cl and also alkaline phosphatase) resulting to the improvement of hepatocyte function, compensate metabolic dysfunction and increase the success rate of IVF by means of improvement in metabolic processes in myocytes and provide OHSS and preclampsia prevention.

Impact of uterine contractile activity on embryo implantation

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Elevated uterine contractility in women undergoing embryo transfer may affect implantation rate. In patients with “silent” uterus, implantation rate seems to be higher as compared to patients with elevated uterine contractility. In animal model, it was confirmed that oxytocin presents a negative effect on implantation. It is obvious that implantation rate differs according to type of COH protocol. The aim of the study was to assess the influence of uterine contractility on embryo implantation rate depends on the type of controlled ovarian stimulation. Two different methods for uterine contractility assessment were also compared in the study.

Material and Methods 2 groups of patients who underwent controlled ovarian stimulation in GnRH agonist and GnRH antagonist protocols were involved to the study. Assessment of uterine contractility was performed using Snake Studio method against M-mode measurement. Visualization of the maximum longitudinal (mid-sagital) plane of the uterus including serosa, myometrium and endometrium was used to assess the influence of uterine contractility on embryo implantation rate depends on the type of controlled ovarian stimulation. Two different methods for uterine contractility assessment were also compared in the study.

Results and discussion Results and discussion

Correlation of uterus contractility with embryo implantation rate was confirmed by ultrasound 4 weeks after embryo transfer. The higher mean level of estradiol in the last day of stimulation was observed in GnRH antagonist protocol. The Snake Studio technique provided more global and accurate measurements, showing overall changes in structure of whole uterine plane. M-mode technique has been highly sensitive to fake-movements, breathing, bowel movements and probe instability.

Conclusions Embryo transfer procedure is an independent factor affecting the success rate. Assessment of uterine activity before transfer can be useful prognostic factor, but it requires additional research studies.
Conclusion: Under pituitary desensitization by nasal administration of GnRH agonist, diminished but significant LH surge can be induced by administration of GnRH agonist at increased dosage within one hour before hCG. This novel protocol significantly improved embryonic development and ongoing pregnancy rate, suggesting critical role of mid-cycle surge of LH and other hormones in oocyte maturation.

FC 15-5 ART clinical

Effect of artificial shrinkage on clinical outcome in fresh blastocyst transfer cycles

Introduction: Artificial shrinkage (AS) before vitrification has been proposed as a useful approach for cryopreservation of blastocysts improving the viability of vitrified-warmed blastocysts. It might help to assist hatching, as well as avoid the formation of ice crystals during freezing. However, there is not enough information as to whether or not AS, in the fresh blastocyst transfer cycles, is necessary to improve the implantation and pregnancy rates. This study was carried out to evaluate the effect of AS on the implantation and pregnancy rates in the fresh blastocyst transfer cycles.

Materials and Methods: The transfers of fresh blastocyst stage embryos from June 2008 to December 2008 were performed in 77 cycles. They were divided into 2 groups: group 1 consisted of the cycles whose intact embryos were transferred (n = 43), while group 2 consisted of the cycles whose embryos were replaced following AS because they were allocated for assisted hatching (n = 34). AS in group 2 was induced under a light stereomicroscope using two needles (29 gauge). The implantation and clinical pregnancy rates were compared between group 1 and 2. Statistical differences were analyzed between group 1 and 2 by Chi² with p < 0.05.

Results: The mean number of transferred embryos in group 1 was 2.2 ± 0.47 (SD), while that in group 2 was 2.0 ± 0.0 (SD). The clinical pregnancy rate in group 2 was significantly higher than that in group 1 (58.8% vs 32.6%). The implantation rate in group 2 was also higher than that of group 1 (34.7% vs 24.0%), but there was no statistical difference between the 2 groups.

Conclusion: These results suggest that AS of blastocoele cavity, followed by the transfer, would be a useful approach to improve the clinical outcome in cycles in which fresh blastocyst stage embryos are transferred. It would be a worthwhile effort to reduce the number of embryos transferred and to increase the probability of single embryo transfer (SET).

FC 15-6 ART clinical

Abnormal first cleavage is a poor prognosis indicator in assisted reproductive technology

Aim: The first cell division of human embryos frequently yields fragmentation. The morphological evaluation of cleavage stage embryo is largely based on the amount of fragments. However, it has been observed that fragments are sometimes fused and absorbed to the blastomere. Moreover, the blastomere without any chromosomal DNA is sometimes found during the preimplantation genetic diagnosis. We aimed to eliminate such abnormal embryos with an extra-large fragment by checking the process of the first cleavage division using the brand-new TLC system.

Method: We developed a new system of a built-in microscope incubator which can produce time-lapse images from up to 90 embryos at one time. Using the system in our IVF program, we analyzed the first cell division data from all the 1,139 cleaved embryos from the series of 282 fresh embryo cycles.

Results: We found abnormal cleavage furrow formation in the first cell division in 312 (27.4%) embryos from 144 (51.1%) cycles. The abnormal cell division produced an extra-large fragment which can be mistakenly recognized as a blastomere after the second cell division. We designated such embryos as crumble embryos. Crumble embryos seemed less capable of embryogenesis. Of 312 crumble embryos, only two embryos reached live birth, one ended in miscarriage, and still four embryos are under cryopreservation. Crumble embryos are significantly observed in the cycles of large number of retrieved oocytes. Odds ratio (OR) for crumble embryos was 1.91 (95% confidence interval (CI): 1.45–2.50) when 15 or more oocytes were retrieved. OR for crumble embryos was 0.67 (95% CI: 0.49–0.91) in the cleomiphene cycle. Crumble embryos were not increased or decreased with aging or ICSI or endometriosis.

Conclusion: The first cell division was shown to be more unstable than that we might think when it was observed using TLC.
Crumble embryos are promising new concept for the embryo evaluation. Crumble embryos provide more direct evidence for cell death rather than fragmentation. This concept is also useful to eliminate the abnormal embryos from the early stage of embryogenesis especially when large numbers of oocytes were retrieved. Further studies are required using our new built-in microscope incubator.

Source of funding of the current study none.

FC 15-7 ART clinical
Supplementation of gonadotrophin releasing hormone (GnRH) agonist during the luteal phase improves the pregnancy outcome in intrauterine insemination (IUI) cycles, when compared with human chorionic gonadotrophin (hCG).

Subjects and Methods
Setting: Tertiary care infertility center between 1.10.09–28.2.10.
Patients: 376 women undergoing IUI cycles treated using LAH for quarter thinning of the zona pellucida and in 44 cases (control group) embryos were thawed with 94% survival rate. Mean conception. Diode Laser ablation has been studied for thinning of the zona pellucida and was known as laser assisted hatching (LAH). Still, there is no sufficient studies recommending routine LAH application. Whereas, Studies has reported a significant increase in pregnancy and implantation rates with assisted hatching of thawed embryos from slow-freezing. The aim of this study was to determine if Laser assisted hatching (LAH) can improve pregnancy and implantation rates for vitrified-thawed embryo transfer cycles.

Methods
Couples enrolled in this study were counseled and consented for vitrification-thawing and laser assisted hatching. A total of 86 vitrified-thawed embryo transfer cycles were randomly assigned to one of 2 groups. In 42 cases (study group) prepared for thawed-cycle transfer, embryos were treated using LAH for quarter thinning of the zona and in 44 cases (control group) embryos were thawed after thawing without any treatment. Vitrification-thawing was performed using Irvine kit™ (USA). LAH was performed using SaturnActive system (RI, UK); 30 min before embryo transfer. Transfer was performed 1–3 hours after thawing. Vitrified embryos were transferred in a soft stimulation protocol cycle.

Results
A total of 298 vitrified embryos were thawed with 94% survival rate. Mean

FC 15-8 ART clinical
Prion Protein Identified in Urinary Gonadotrophin Preparations

Introduction
Ur ine-derived gonadotrophins contain several impurities. Lately, prion protein has been identified in a preparation of human menopausal gonadotrophin (hMG). This new finding is due to the employment of a more sensitive Mass Spectrometry (MS) method. The present study was aimed at assessing the presence of prion proteins in urinary and recombinant human chorionic gonadotrophin (hCG) preparations by using advanced proteomic analyses.

Methods
Recombinant-hCG and two urinary hCG preparations (products A and B) were initially subjected to 2-dimensional (2D) gel electrophoresis followed by proteomic analysis by Liquid Chromatography-Mass Spectrometry (LC-MS/MS). In a second step, a prion targeted MS analysis by Multiple Reaction Monitoring (LC-MRM) was carried out.

Results
Several non-gonadotrophin proteins were identified by the classical proteomic protocol in the urine derived hCG preparations. No contaminants were identified in the recombinant preparation. Within urinary impurities, prion protein (Swissprot accession number P04156) was identified in several 2D gel spots in the mass range between 20–25 kDa in product A (four spots at 25 kDa and several at lower molecular masses) and at about 29 kDa in product B (3 spots). An LC-MRM analysis was performed directly on the samples without any separation. 4 tryptic prion peptides were specifically detected confirming the presence of a prion protein in urinary-derived hCG samples.

Conclusions
Using classical and targeted proteomic analyses, the prion protein was detected for the first time in two urinary hCG preparations but not in the recombinant hCG preparation. This is the first report of the presence of prion proteins in commercial urinary hCG preparations. The prion protein was identified in several spots, likely due to heterogeneous glycosylation and degraded forms. These findings indicate that different purification processes for different urinary-derived preparations are unable to remove prion proteins from the source material and the process controls employed do not permit the identification of this impurity. To date, there is no evidence that prion-related disease can be acquired by using urinary gonadotrophins, however the potential risk should be reconsidered.

Funding source
Merck Serono GmbH (an affiliate of Merck KGaA, Darmstadt, Germany)

FC 16-1 ART outcome
Laser assisted hatching in vitrified-thawed embryos

Introduction
Vitrification became widely applied to substitute slow-freezing, after evidences of higher survival and pregnancy rate. Hardening within zona pellucida has been reported with slow freezing cryopreservation. This observation might explain inability of cleaving blastocyst to escape from the zona pellucida resulting in failure of implantation and conception. Diode Laser ablation has been studied for thinning of the zona pellucida and was known as laser assisted hatching (LAH). Still, there is no sufficient studies recommending routine LAH application. Whereas, Studies has reported a significant increase in pregnancy and implantation rates with assisted hatching of thawed embryos from slow-freezing. The aim of this study was to determine if Laser assisted hatching (LAH) can improve pregnancy and implantation rates for vitrified-thawed embryo transfer cycles.

Methods
Couples enrolled in this study were counseled and consented for vitrification-thawing and laser assisted hatching. A total of 86 vitrified-thawed embryo transfer cycles were randomly assigned to one of 2 groups. In 42 cases (study group) prepared for thawed-cycle transfer, embryos were treated using LAH for quarter thinning of the zona and in 44 cases (control group) embryos were thawed after thawing without any treatment. Vitrification-thawing was performed using Irvine kit™ (USA). LAH was performed using SaturnActive system (RI, UK); 30 min before embryo transfer. Transfer was performed 1–3 hours after thawing. Vitrified embryos were transferred in a soft stimulation protocol cycle.

Results
A total of 298 vitrified embryos were thawed with 94% survival rate. Mean
number of surviving thawed embryos for transfer was 3.25 ± 1.9 and were transferred in 86 cycles. In 42 cycles, thawed embryos were treated using the LAH and in 44 cycles where vitrified embryos transferred were not used for LAH. It has been found that there was no significant differences in the rates of pregnancy (33.3% vs 45.5%), or implantation (14.1% vs 13.9%) between the LAH treated embryos and the embryos transferred without LAH respectively.

**Conclusions**

Our results confirm vitrification high survival and pregnancy rates. An acceptable pregnancy rate was obtained from laser treated thawed embryos transfer for assisted hatching, still our results did not show any significant change in pregnancy rates.

**FC 16-2 ART outcome**

**Role of double IUI in cases with high Pre-hCG PSV**

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**Aim**

The aim of the study was to find out if double IUI can increase the pregnancy rate in patients who have a pre-hCG perifollicular PSV > 15 cms/sec.

**Background**

It has been established that optimum perifollicular PSV, on the day of hCG, has been confirmed as > 10cms/sec. Another study also says that the perifollicular PSV of 42 cms/sec is reached about an hour before the spontaneous rupture of the follicle. This means that if the follicle is said to be functionally mature when PSV is 10cms/sec, that is the time when the LH surge starts and under the effect of that LH, the perifollicular PSV keeps on rising constantly. That means if higher the PSV, the follicle is closer to rupture. Keeping this in mind, a study was done taking all those patients who had perifollicular PSV of > 15 cms/sec on the day of hCG.

**Method**

300 IUI cycles were studied, which included the patients stimulated with clomiphene citrate, rFSH and letrozole + rFSH. As per the standard protocols, the pre-hCG parameters selected for follicle and endometrium.

- hCG 10,000 was given for ovulation trigger.
- For all the patients with perifollicular PSV > 15 cms/sec, single or double IUI was done randomly.
- They were grouped into a PSV of 15–20, 20–25 and > 25 cms/sec.
- Single IUI was done between 36 and 38 hours and double IUI were done at 12–14 hours and 36–38 hours.
- Cases with wash count between 7 and 10 million/ml were selected for the study.
- The results of the cycles were studied. Conception was considered as a desired result and nonconception as an undesired result.

**Results**

In all protocols, pregnancy rates were significantly high when an early IUI was added in patients with perifollicular PSV > 20 cms/sec on the day of hCG.

**Conclusion**

With any protocol when perifollicular PSV on the day of hCG is > 25 cms/sec, double IUI must be done. With rFSH and combination protocols with the PSV values > 20 cms/sec also double IUI must be preferred. With PSV < 20 for any protocol, single and double IUI show no significant change in pregnancy rates.

**FC 16-3 ART outcome**

**Safety and viability of the vitrification technique for an oocyte donor bank program – experience of 3 years**

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**Aims**

Vitrification may become a routine in Assisted Reproductive Technologies (ART), especially as an alternative of traditional donor programs, solving the long waiting lists, creating a donor catalog, reduction in number of freezing spare embryos and lowering the economical costs, but with same result as fresh programs.

The aim of our study was to show that oocyte vitrification and the sequential culture are a safe combination to have a viable success in an oocyte donation bank program.

**Methods**

30 cycles of oocytes donors were frozen by vitrification. We study 16 patients cycles of thawed oocytes receptors, done from May 2006 to December 2009. As a control group we included 48 patients cycles of fresh oocyte receptors. Oocytes were retrieved and vitrified with the Cryotop method with 7.5 % ethylene glycol, 7.5 % propanediol, and 0.5 mol/L sucrose. 154 oocytes were thawed, and mean of 9.6 oocytes MII were retrieved was similar between IVM and IVF cycles. In IVM cycles after in vitro culture, development of frozen oocytes after ART. Vitrification may become a routine in Assisted Reproductive Technologies (ART), especially as an alternative of traditional donor programs, solving the long waiting lists, creating a donor catalog, reduction in number of freezing spare embryos and lowering the economical costs, but with same result as fresh programs.

The statistical analysis was performed by SPSS software and Mann-Whitney U-tests were carried out. Significance was defined as p < 0.05.

**Results**

The oocyte survival, fertilization, day 2 to cleavage, day 3 cleavage and blastocyst development rates were: 96.75%, 85.71%, 93.9%, 77.41%, and 41.66% respectively. A total of 29 embryos were transferred on blastocyst stage. The media of embryos transferred were 2.

The refreezing rate of spare embryos by vitrification was 27.5%. Pregnancy, implantation and miscarriage rates, were 66.66%, 31.06% and 22.22%, respectively. Our pregnancy, implantation and miscarriage rates in fresh egg donation were 70.55%, 36.11% and 13.04% respectively. No statistical differences were found between both groups.

**Conclusions**

Our excellent clinical outcome indicates the safety to use the vitrification to create oocyte donation bank programs for human reproductive purposes. Sequential culture is a viable strategy to consider to select embryos with a high potential implantation to transfer. More studies should be done to confirm these results and to understand better the oocyte physiological process after thawing and improve the survival and further development of frozen oocytes after ART.
Introduction Intrauterine insemination with frozen donor sperm (IUI-D) is a method of treatment in women with azoospermic partners. Assuming that the women requiring IUI-D are fertile, the success of the treatment is influenced by a number of variables including the controlled ovarian stimulation (COS). The aim of the study was to compare the pregnancy outcome of IUI-D following COS with or without gonadal stimulation (FSH, HMG), aromatase inhibitors (AI) versus spontaneous cycle (SC).

Materials and Methods A retrospective study of all the patients who underwent IUI-D between 01.01.2005 and 31.12.2009 in our unit was performed. A total of 108 couples and 334 IUI-D treatment cycles were analyzed. The following parameters were measured: type of hormonal treatment, number of follicles, female age, semen quality, term pregnancies were induced in cycles with higher concentration of motile spermatozoa (2.88 vs 1.77 millions (p < 0.001)).

Conclusions The results show that SC gives better results in terms of pregnancy rates than any of the considered COS cycles when a unifollicular response to avoid multiple pregnancies is intended. The variable that influenced the pregnancy outcome following IUI-D in our study was the quality of the donor sperm after thawing.

Clinical experience may further improve the results.

FC 16-5 ART clinical Impact of different ovarian stimulation protocols on the pregnancy outcome of intrauterine insemination with frozen donor sperm

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Introduction Intrauterine insemination with frozen donor sperm (IUI-D) is a method of treatment in women with azoospermic partners. Assuming that the women requiring IUI-D are fertile, the success of the treatment is influenced by a number of variables including the controlled ovarian stimulation (COS). The aim of the study was to compare the pregnancy outcome of IUI-D following COS with or without gonadal stimulation (FSH, HMG), aromatase inhibitors (AI) versus spontaneous cycle (SC).

Materials and Methods A retrospective study of all the patients who underwent IUI-D between 01.01.2005 and 31.12.2009 in our unit was performed. A total of 108 couples and 334 IUI-D treatment cycles were analyzed. The following parameters were measured: type of hormonal treatment, number of follicles, female age, semen quality, term pregnancy rate, cumulative pregnancy rate, miscarriage rate and multiple gestations rate. Results COS was performed using CC in 32 cycles (9.6%), gonadotrophins in 168 cycles (50.3%), CC-HMG in 5 cycles (1.5%), AI-FSH/HMG in 31 cycles (9.3%), and SC were used in 98 cycles (29.3%). Pregnancy rates were 13.3% with CC, 20.0% with gonadotrophins, 40.0% with CC-HMG, 14.3% with AI-FSH/HMG, and 12.6% in SC. The overall clinical pregnancy rate per cycle was 5.9%. The overall miscarriage rate was 3.2% and the overall baby take-home rate was 13.4%. The overall cumulative pregnancy rate per couple was 49.0%. The mean number of follicles obtained in all cycles was 1.2 as was the mean number of follicles obtained in patients with the cycles leading to pregnancy. There was a statistically significant difference between the mean number of follicles observed in the stimulated cycles compared to SC (1.4 and 1.02 respectively; p = 0.008). However, there was no statistical difference between the rate of pregnancies of stimulated cycles versus spontaneous cycles (p = 0.18). The mean concentration of motile spermatozoa after washing was 1.96 millions on the day of IUI-D for all cycles, but pregnancies were induced in cycles with higher concentrations of motile spermatozoa (2.88 vs 1.77 millions (p < 0.001)).

Conclusions The results show that SC gives better results in terms of pregnancy rates than any of the considered COS cycles when a unifollicular response to avoid multiple pregnancies is intended. The variable that influenced the pregnancy outcome following IUI-D in our study was the quality of the donor sperm after thawing.

FC 16-6 ART clinical Intrauterine tuboperitoneal insemination (IUTPI) – a new method of intrauterine insemination experience after 1000 cycles

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Introduction Intrauterine Tuboperitoneal Insemination (IUTPI) was firstly introduced in 2002. It is a novel intrauterine insemination technique that utilizes 10 ml of insemination compared to 4 ml used in Fallopian Sperm Perfusion (FSP) or 0.5 ml used in standard Intrauterine Insemination (IUI). The larger volume of inseminate used in IUTPI is believed to be able to overcome the intrauterine pressure at the level of the tubal ostia. This information was combined with the higher live birth rates in patients with unexplained infertility following hysterosalpingography, a technique which also utilises 10 ml of contrast medium. The aim of this retrospective clinical study is to evaluate the effectiveness of the use of 10 ml of inseminate in pregnancy rates following IUTPI.

Materials and Methods From 2002 to 2009, 586 couples with unexplained infertility, mild to moderate male infertility and mild or moderate endometriosis underwent 1028 cycles of IUTPI. Women were younger than 42 years old. The mean inseminate motile count (IMC) following sperm preparation was 17.02 ± 13.5 (range from 2 to 80 million, motility 60–80%). All women followed the same ovulation induction protocol consisting of clomiphene citrate (CC) from day 2 to day 6 followed by 75 IU or 150 IU of hMG from day 6, depending on the age and BMI of the patient. A transvaginal ultrasound scan along with E₂ and LH measurements were performed on day 10 of the cycle. Ovulation was triggered with 5,000 IU of hCG when the leading follicle reached 18 mm in diameter. Luteal phase was supported with progesterone. Clinical pregnancy was confirmed when an intrauterine gestational sac was visible on ultrasound scanning.

Results In the 1028 cycles performed in our Center, 272 pregnancies were achieved out of 11 in frozen-thawed cycles. The endometrial thickness in hormone supplemented cycles increased significantly after LA (from 8.9 mm to 10.3 mm; p = 0.01), but not in fresh cycles (from 10.3 mm to 11.1 mm). LA did not show any adverse effects on either laboratory or clinical parameters.

Conclusions LA treatment improved not only laboratory aspects of IVF, but also endometrial environment. The present study implied that LA should be applied on any IVF failures as an integrated medicine not only because of their benefit, but also no adverse effect.

FC 16-7 ART clinical Effects of laser acupuncture (LA) on multiple in vitro fertilization (IVF) failures

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Introduction Assisted reproductive technology (ART) has shown advancements in recent and could be effective for many infertility patients. However, there are few solutions to poor responders and multiple in vitro fertilization (IVF) failures due to increase of aged patients. In the present study, the effects of laser acupuncture (LA) on IVF treatment were evaluated on multiple IVF failures.

Materials and Methods Retrospective analysis was performed on 48 patients undergoing IVF (204 cycles) from July 2008 to February 2010. The laser device which emits wave length of 830 nm with 60 mw was used. Laser was first irradiated to the neck area for 10 min while the therapist stretches the neck and shoulders to increase brain and systemic blood flow. Then irradiation was focused on reproductive organs for 5 min to increase blood circulations. Patients received LA once a week for at least 3 times. Mean age of the patients was 42.0 and they got LA 3 to 34 times. Every aspect of IVF was compared before and after LA treatment.

Results 10 patients out of 48 conceived successfully after LA. Several parameters were significantly improved after LA as follows. Number of oocytes retrieved (from 2.8 to 3.8; p = 0.03), matured oocytes (from 2.3 to 3.3; p = 0.02), oocytes fertilized (from 2.1 to 2.7; p = 0.02), embryos frozen (from 1.5 to 2.3; p = 0.03) and good quality embryos (from 1.8 to 2.8; p = 0.04). 4 patients out of 10 conceived in fresh cycles and 3 patients out of 11 in frozen-thawed cycles. The endometrial thickness in hormone supplemented cycles increased significantly after LA (from 8.9 mm to 10.3 mm; p = 0.01), but not in fresh cycles (from 10.3 mm to 11.1 mm). LA did not show any adverse effects on either laboratory or clinical parameters.

Conclusions LA treatment improved not only laboratory aspects of IVF, but also endometrial environment. The present study implied that LA should be applied on any IVF failures as an integrated medicine not only because of their benefit, but also no adverse effect.

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IFFS 2010 – Oral Abstracts
FC 17-1 ART outcome

Obstetric haemorrhage after ART: An analysis of 6730 singleton births

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Aim Obstetric haemorrhages have been reported to be increased after ART. The aim was to confirm this and study the mechanisms.

Methods This retrospective cohort study compared the frequencies of antepartum haemorrhage (APH), placenta praevia (PP), placental abruption (PA) and primary post-partum haemorrhage (PPH) for women with singleton births between 1991 and 2004: 6730 after IVF/ICSI, 779 after gamete intra-fallopian transfer (GIFT), 2167 non-ART conceptions in infertility patients and 24619 from the general population.

Results The IVF/ICSI group had more APH: 6.7% vs 3.6% (adjusted OR 2.0; 95%-CI: 1.8–2.3), PP: 2.6% vs 1.1% (2.3; 1.9–2.9), PA: 0.9% vs 0.4% (2.1; 1.4–3.0) and PPH: 11.1% vs 7.9% (1.3; 1.2–1.4) than the population controls. PPH, PA and PP were as frequent in the GIFT group but less frequent in the non-ART group. Within the IVF/ICSI group, fresh compared with frozen thawed embryo transfer (FET) was associated with more frequent APH (1.5; 1.2–1.8) and PA (2.1; 1.2 to 3.7) and the odds ratio increased with the number of oocytes collected (1.02; 1.00–1.04). Patients with endometriosis had more PP (1.7; 1.2–2.4) and PPH (1.3; 1.1–1.6). When hCG was used for luteal phase support there was less PPH (0.7; 0.6–0.9). FET in artificial cycles was associated with increased PPH (1.8; 1.3–2.6).

Conclusion Some ART related factors increase the frequency of obstetric haemorrhages with singleton births. The exploratory analysis of factors in the IVF/ICSI group showing associations with fresh transfers in stimulated cycles, hormone treatments and endometriosis suggests events about the time of implantation may be responsible and that suboptimal endometrial function is the critical mechanism.

FC 17-2 ART outcome

Perinatal outcomes of assisted reproductive technology in endometriosis

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We aimed to investigate perinatal outcomes following Assisted Reproductive Technology (ART) in women with endometriosis and ovarian endometriosis using a retrospective cohort study of 4,387 randomly selected Victorian mothers of singleton babies, including ART patients, subfertile and fertile women. 630 ART patients had endometriosis, of which 95 had ovarian endometriosis. 1,201 ART patients with other aetiologies of infertility and 156 subfertile women, who were treated for ART treatment but conceived naturally, were also included. 2,400 age-matched fertile controls were selected from a database of all 850,000 Victorian births from 1991–2004. These were selected for comparison with women with any form of endometriosis and those with ovarian endometriosis. Ovarian endometriosis was diagnosed by at least 2 positive pelvic ultrasounds. Other forms of endometriosis were diagnosed by laparoscopic/histologic reports. ART patients with Ovarian endometriosis were compared with ART patients with other forms of endometriosis, ART patients with other aetiologies of infertility, subfertile and fertile controls; and ART patients with any form of endometriosis were compared with ART patients with other aetiologies of infertility, subfertile and fertile controls. Adjusted odds ratios (AOR) were calculated using binomial logistic regression with covariates including parity, maternal age, smoking status and sex of baby. The likelihood of low birthweight (LBW) ART babies from mothers with ovarian endometriosis was increased compared with other aetiologies. The overall AOR was 2.87 (95%-CI: 1.49–5.55; p = 0.002), and 2.55 (95%-CI: 1.26–5.18; p = 0.009) when compared with non-ovarian endometriosis.

Babies of ART patients with ovarian endometriosis also had a higher likelihood of being small for gestational age (SGA) compared with those from ART patients with other forms of endometriosis (AOR 1.20, 95%-CI: 1.04–3.83; p = 0.04). Additionally, there was an increased likelihood of having a LBW baby for ART ovarian endometriosis patients when compared with subfertile patients (AOR 2.29, 95%-CI: 1.02–5.16; p = 0.05).

Women with ovarian endometriosis who have ART are at greater risk of having a LBW or SGA baby compared with other forms of endometriosis. Ovarian endometriosis increased LBW risk compared with other aetiologies of infertility following ART and compared with subfertile patients. This study has found that women with ovarian endometriosis have smaller babies than other women following ART.

FC 16-8 ART clinical

In vitro maturation: 5-year experience

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Introduction Although several patients may benefit from reduced FSH application, in vitro maturation (IVM) belongs to the rare treatment options in assisted reproduction. In this study, we present our 5-year experience with IVM at our department of gynaecological endocrinology and fertility disorders.

Materials and Methods Women with polycystic ovary syndrome (PCOS) after unsuccessful ovarian stimulation for IVF/ICSI with ovarian hyperstimulation syndrome (OHSS) 2–3 were offered IVM. Stimulation started between day 3 and 10 of the menstrual cycle and FSH application was 125 IU/day for 3 days. Ovulation was induced on the third day of FSH injection or one day after. Oocyte retrieval was performed 33–38 hours later and oocytes were cultivated for up to 24 hours in IVF medium. Fertilisation took place one day after oocyte retrieval and embryo transfer 2 days afterwards. Embryos were scored as followed: grade A = 4, grade B = 3, grade C = 2, grade D = 1 point.

Results From February 2005 until December 2009 115 patients were included with 215 oocyte retrievals and 177 embryo transfers. Mean age of the patients was 30.8 ± 4.3 years and BMI was 24.6 ± 5.5 kg/m². FSH levels on Day 3–5 were 5.6 ± 1.9 and estradiol levels 75.7 ± 110.5 pg/ml. Main reasons for IVM were: PCOS (64.6%) and OHSS (15.0%). In 125 cases IC50 (59%) and in 73 IVF (34.4%) was performed. Mean number of oocytes was 8.9/oocyte retrieval with 5.9 becoming matured, 2.8 getting fertilized and 2.1 being transferred as embryos. The mean embryo score was 15.8 ± 10.8. Pregnancy rate per transfer was 15.3% (n = 27), with n = 12 live births (6.8%), n = 2 ongoing pregnancies (1.1%), n = 4 abortions (2.3%) and n = 9 biochemical pregnancies (5.1%). In 61 cases, fertilized oocytes were frozen and 32 cryotransfers were performed resulting in 3 pregnancies (1 biochemical, 1 live birth, 1 ongoing pregnancy).

Summary Within the last 5 years, 115 patients with PCOS or OHSS 2–3 were included in our department. Pregnancy rate was 15.3%, 7.4% ended up in abortion or biochemical pregnancies. Nevertheless, IVM should be offered for patients after OHSS or unsuccessful controlled ovarian stimulation (PCOS). Furthermore, IVM is very comfortable for patients due to low FSH dosages implicating low cost rates and only one appointment before oocyte retrieval.

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Perinatology Academician VI Kulakov, Moscow; these patients contributes to further second
OHSS that could predetermine high risk for
dence of chorion pathology in patients with
Conclusions
of the early neonatology period were analyzed.
Methods
IVF pregnancies in women with severe OHSS.
Objective
emis without OHSS. The course of the first,
several cases of third trimester of IVF pregnan-
acies, pregnancy outcomes and characteristics of the early neonatology period were analyzed.
Results
In the first trimester of pregnancy after OHSS low placentaion was found in
45.5% women (26.7% in group 2). Incidence of chorion hypoplasia and deciduitis (30.3%) was
4.5-fold higher in group 1 than in group 2 (6.7%), chorion abruption before 14 weeks of gestation occurred only in group 1, “van-
ishing twin” syndrome in case of multiple pregnancy was 2.5-fold more prevalent in patients with OHSS (16.7%) than in group 2
(6.7%). Incidence of early pregnancy loss was 3-fold higher in group 1 than in group 2.
The most common complication in the sec-
ond trimester was threatened abortion. The second and the third most common compli-
cations were amenia (29.1%) and istmic-
cervical insufficiency (25.5%) respectively, the latter associated with multiple pregnancy in
3.7%. Incidence of placental abrasion was 2.5-fold higher in group 1. In the third trimester 29% of women in group 1
were diagnosed with impaired fetoplacental circulation, 16% with fetal growth restric-
tion, 16% with intrauterine hypoaxia and 12% with preeclampsia. Term delivery rate was
47.1% and 76.7% in group 1 and group 2, respectively, preterm delivery rate – 22.9% and
13.3%, respectively. Incidence of preg-
nancy loss in the first and the second trimer-
ter was 2.5-fold higher in patients with OHSS than in group without OHSS (17.2% and
7.1%, respectively). 41.7% of all children were born prematurely. Incidence of infec-
tious diseases and respiratory disorders in new-
borns was 44% and 36.4%, respectively and was positively correlated with age of gestation.
Conclusions
significant higher inci-
dence of chorion pathology in patients with
OHSS that could predetermine high risk for
early pregnancy loss was found. It is likely that the abnormal first trimester course in these patients contributes to further second and third trimester pregnancy complications, including IUGR, preeclampsia, perireth birth and, finally, perinatal complications.
FC 17-4 ART complications
Color doppler ultrasound guided oocyte retrieval in women with adnexal varicocities
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Internal and external bleeding occurs in 2–3% of patients following oocyte aspiration. It has been suggested that enhanced visualization of parametral blood vessels using color Doppler ultrasound may reduce peritoneal and vaginal bleeding. The purpose of this study was to identify a high risk for bleeding patient subpopulation and to assess the possible impact of Doppler ultrasound on peritoneal bleeding.
Materials and Methods
Oocyte aspiration was performed in 1076 women in Caracas, Venezuela between December 2006 and March 2010. The data was independently evaluated in Chicago, Illinois, USA. Three or more Doppler enhanced parametral blood vessels defined adnexal varicocities. Moderate to severe peritoneal bleeding was recorded in the pres-
ence of more than 3 cm large fluid filled pockets.
Results
Moderate to severe peritoneal bleeding occurred in 52/1076 (5%) of the pa-
tients. Moderate to severe peritoneal bleeding was detected in 18/1008 (2%) patients without adnexal varicocities compared to 34/
68 (50%) patients with adnexal varicocities (p < 0.05). Pelvic endometriosis was present in 10/68 (15%) of patients with adnexal vari-
cocities and 4/10 retrievals resulted in moder-
ate to severe peritoneal bleeding. Adnexal varicocities were identified along the needle trajectory in all 34/68 patients with moderate to severe peritoneal bleeding.
Conclusions
Patients with adnexal varicocities detected by color Doppler ultrasound have a 25 fold risk of moderate to severe peritoneal bleeding following oocyte aspiration. Pelvic adhesions may reduce peritoneal bleeding in patients with adnexal varicocities. These data raise pertinent questions on withholding unilateral or bilateral oocyte as-
piration in patients with adnexal varicocities and the selection of a safe needle trajectory during oocyte aspiration.
FC 17-5 Economic issues
Medicinal aspects of reasonable re-
productive health policy
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IVF and other assisted reproductive tech-
niques are now available in most countries. By now, most European nations have adopted laws to regulate assisted reproduction. These laws reflect a variety of cultural, religious, political, and economic values and prefer-
ences testify to the complex nature of as-
sisted reproduction. Countries also vary in the intensity of regulation and their accep-
tance of the most advanced techniques such as egg donation and reproduction with donors. Some countries have strict legal prohibitions, others require prior regulatory approvals, and still others leave it to the discretion of the physician and patient. As a result, there are important differences among nations con-
cerning the terms and conditions of their use, including differences in such important is-
ues as the number of embryos created and transferred, how they are stored and their fi-
nal disposition. These legal regulations do have an important impact on practice, most notably with regard to the efficacy and safety of treatments. The main areas of restrictions of law are related to the limitation of the number of oocytes allowed to be fertilized, number of transferred embryos and embryos cryopreservation policy. Restrictive law implemented in Italy and Germany resulted in serious consequences for all parties in-
volved in infertility treatment, with specially negative impact on patients who pay a price in terms of loss of efficiency and increase of risks of medical complications. Fertilizing just three oocytes will cut the success rates by about two thirds. Also limitation in num-
ber of transferred embryos to one (SEET) in unselected patients will halve the pregnancy rate compared with double-embryo transfer. The ability to preserve embryos for future use has brought two major benefits to the in-
fertile couple. Because of no need to repeat the entire IVF cycle, the cost is lowered. Sec-
ondly, after a month or more recovery period, the woman’s natural menstrual cycle can be used to determine the optimum time for em-
bryo transfer when the woman’s uterus is natu-
rally ready for implantation. The embryo cryo-
preservation ban is thus really harmful for patients specially when there is no reimburse-
ment policies. From professional perspective the most important challenge is to assure that patients receive the best possible treatment and are not exposed to unnecessary risks.

FC 17-6 ART outcome
A novel classification and analysis of birth defects in babies conceived using assisted reproduction
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versity, Department of Obstetrics and Gynaecology, Clayton; 1Monash Childrens Research Institute, Clinical Genetics Research, Parkville, Australia
Introduction Babies conceived using as-
sisted reproduction technologies (ART) have a 30% increased likelihood of being born with a birth defect, however causative path-
ways are unknown. Previous studies have
grouped birth defects in ART using anatomical classifications (ICD-9), potentially masking aetiological information. We hypothesized that greater insight into the aetiology of birth defects in ART could be gained by using a classification based on aetiology.

Methods We examined all birth defects in a cohort of 6,946 ART singleton conceptions and 20,838 non-ART controls from a retrospective record linkage cohort study covering the years 1991-2004. All birth defects were reclassified using a hierarchical system comprising seven aetiological categories. Birth defects of unknown aetiology were further classified according to whether the embryological origin of the birth defect(s) could be traced to blastogenesis, the first four weeks of pregnancy.

Results Overall, birth defects were increased in ART pregnancies (adjusted odds ratio [adjOR] 1.36; 95%-CI: 1.19–1.55) relative to controls, but there was no strong evidence of risk differences between IVF and ICSI or between fresh and thawed embryo transfer (ET). Compared to non-ART controls, ART babies were no more likely to have a birth defect of unknown genetic aetiology (adjOR 0.89; 95%-CI: 0.51–1.56) or chromosomal abnormality (adjOR 1.09; 95%-CI: 0.71–1.68). ART babies were however, more likely to have defects of blastogenesis (adjOR 2.80; 95%-CI: 1.63–4.81) compared to non-ART controls. Further, for blastogenesis defects, the increase relative to controls was significant for fresh ET (adjusted OR 3.65; 95%-CI: 2.02 to 6.59) but not for thawed ET (adjusted OR 1.60; 95%-CI: 0.69–3.69).

Conclusions Our data suggest that the increase in birth defects in ART pregnancies is the result of an increase in blastogenesis defects rather than known genetic defects, and supports the hypothesis that this may be due to environmental or epigenetic factors resulting from the ART process, particularly those associated with fresh ET. Having recoded all birth defects in the Victorian Birth Defects Register according to this new classification, we are now undertaking a case control study to confirm this finding.

FC 18-ART clinical IV

FC 17-8 ART clinical

Mini-IVF and Natural-Cycle-IVF. What’s the hype about it?

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During the last years scientific and public awareness raised about mild, safe, more physiological and affordable approaches in assisted reproduction, namely Natural Cycle IVF and Mild IVF.

Natural Cycle IVF is performed when a single naturally selected oocyte is retrieved in a women’s natural cycle – without any ovarian stimulation.

Mild IVF includes small doses of ovarian stimulation tablets or injections without the suppression of ovaries. The aim is to obtain a small number of oocytes (between 2 and 7) and embryos.

The most widely used protocols in reproductive medicine within the last 15 years was “conventional” IVF in the so-called long-protoocol, where the ovaries of the patient are suppressed for 10–4 days prior to controlled hyperstimulation of ovaries to produce a high number of oocytes and embryos. These protocols are usually time consuming, expensive and associated with considerable patient discomfort and risks of complications – e.g. the ovarian hyperstimulation syndrome. Moreover there is increasing evidence that such stimulation protocols can harm embryo quality, endometrial receptivity and implantation. Some authors emphasize that the number of capable embryos will not rise with the number of retrieved oocytes. And studies revealed also that mild stimulation IVF results in a higher proportion of chromosomally normal embryos.

Since in most countries patients or health insurance companies pay per cycle and centers are usually compared on the basis of pregnancy rates per cycle the stimulation protocols used presently may result in higher pregnancy rates at a first glance but may also result in increased perinatal morbidity and mortality, multiple pregnancy, fetal reduction, enormous cost and human suffering.

Mild approaches to ovarian stimulation promise to be a more science based and patient-friendly IVF. Possibly even may lead to improved health of the offspring.

Whenever planning assisted reproduction treatment the aim should be to take the welfare of the woman and the child into account.

Mild approaches in IVF with the implementation of single embryo transfer appear to be the way forward – safe and cost-effective.

The presentation will discuss pros and cons of these methods according to recent literature.

FC 18-1 Economic issues

Business aspects of an ART center

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“Demography is destiny” (Comte) and Europe shows fertility rates below replacement levels [Gant et al, 2006]. Although policy makers often address infertility treatment in health policies, the genesis of “fertility tourism” is indispensible and powered by a niche which sees in the EU enlargement increased treatment opportunities. This again creates business opportunities for aspiring entrepreneurs, who are often medical professionals who feel encouraged to open an ART Center.

This paper looks at the business opportunities arising from fertility tourism and aims adumbrating the profiles of entrepreneurs of smaller and mid-sized ART Centers; identifying some of the most common pitfalls in running an ART Center and; suggesting ways to avoid them.

A few of Czechia’s ART Centers were analyzed taking into consideration key business
Aspects as Ownership and management structure, Business Strategy and Quality and Risk management and were compared to similar sized private clinics in other European countries, among other UK, Germany, Italy and Greece.

Key findings confirm the assumption that smaller centres managed by owners-physicians are mostly based on the tacit knowledge of their owners and experience a longer learning curve in applying the rules of doing business. Subsequently, despite medical excellence, these centres need to identify mechanisms which will prevent them from missing opportunities for growth and innovation and will ensure that they will remain competitive.

Rather than identifying or emphasizing known facts, the novelty of this presentation lies in putting some key business aspects of an ART Center in a nutshell and providing hands-on suggestions for smaller and mid-sized centers for avoiding the most common pitfalls.

Source of funding Author of the paper.

FC 18-2 Economic issues
Aims of reproductive health policy
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Legislation relating to the assisted reproduction and certain problems of heavy ethical and moral load gives as so many questions about the relation between the ethics and the law. One of them is: How the law could solve moral conflicts in today’s times, times of post-modernistic society characterized by miscellaneous groups of the society with various moral attitudes? The essence of this problem lies in fact at the heart of democratic society. The great influence on human reproduction and biotechnology has created many ethical and moral problems (or controversies) and has evoked world discussion about admissibility of individual procedures and affirmation of the safest way for patients and their families.

In the last 20 years in many countries they enacted special law regulations and acts and EU has processed directives for all the European members of the minimal organizational and legal conditions of the high quality service related to the employment of the human cells, tissues and organs.

The legislation process in the most of democratic societies is not created without the influence of the history, culture and predominating outlook on life as well as religion. Although political reality and informal influences of the parties and social groups for passing the resolutions in many countries with the same democratic system and structure could create completely different law in the aspect of the principle rule and pragmatism. It seems that only international exchange of experience related to (or bind up with) the functioning of the reproductive medicine in the specific legal models and serious discussion among politicians, legislators, professionals and first of all patients could place them into the legal regulations and will be the basis for enacting of modern and fair law.

FC 18-3 ART biology
The effect of the season on human assisted reproduction outcomes
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Introduction Epidemiologists have demonstrated a seasonal distribution in human natural conception and birth rates. Temperatures and seasons have been suggested as environmental factors that influence fecundity in mammals. The present study evaluated whether seasonality affects human assisted reproduction treatments outcomes.

Materials and Methods For this study 1932 patients undergoing oocyte retrieval for intracytoplasmic sperm injection (ICSI) were assigned to a season group according to the day of oocyte retrieval: winter (n = 435, 22.5%), spring (n = 444, 23.0%), summer (n = 469, 24.2%) and autumn (n = 584 30.3%). The percentage of retrieved metaphase II (MII) oocytes and high-quality embryos, fertilization, implantation and pregnancy rates were compared among the groups. Moreover the serum estradiol concentration was compared and, in order to identify the effenences of the ovarian response, the estradiol concentration per number of MII oocytes was also evaluated.

Results The percentage of MII oocytes, high-quality embryos, implantation, and pregnancy rates did not differ among the groups. Nevertheless the fertilization rate was significantly higher during the spring than at any other season (winter: 67.9%; spring: 73.5%; summer: 68.7% and autumn: 69.0%; p < 0.01). In fact a nearly one and a half-fold increase in the fertilization rate during the spring was noted (OR: 1.45, p < 0.01).

No influence of any other season on the fertilization and percentage of high-quality embryos was noted. The serum estradiol was equal among the groups, however, the estradiol concentration per number of MII retrieved oocytes was significantly higher during the spring (winter: 235.8; spring: 282.1; summer: 226.1 and autumn: 228.7; p = 0.030).

Conclusions This study demonstrates a significant seasonal variability in the fertilization after ICSI, where the fertilization is higher during the spring that at any other time. The effectiveness of the ovarian response to the treatment was also higher during the spring in comparison to the other seasons. It has been previously suggested that fecundity in mammals is dependent on daylight and temperature and increasing day length is associated with changes in the brain that are responsible for mediating reproductive activities. Spring, with a long photoperiod, may be associated with increased pituitary-ovarian axis activity in human, leading to the production of high quality gametes, with higher fertilization potential.

Funding none.

FC 18-4 ART biology
Diminished fertility in poor responder young patients is not associated with oocyte morphological status
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Introduction Despite several definitions of poor response, it is still unclear whether young poor responder patients show also a decrease on oocyte quality. The aim of this study was to evaluate the relationship between ovarian response and oocyte quality declining in women younger than 35 years old.

Methods Intracytoplasmic sperm injection (ICSI) cycles performed in patients younger than 35 years old, excepting male infertility cases, were analyzed. Patients who produced four or less metaphase II (MII) oocytes (poor-responder, PR group, n = 57) after ovarian stimulation were age matched with normoresponder patients in which five or more oocytes were retrieved (NR group, n = 256). Groups were compared regarding:

1. Age
2. Oocyte yield
3. MII oocyte rate
4. Percentage of extracytoplasmic and intracytoplasmic oocyte abnormalities; and

ICSI outcome

Results Increased doses of gonadotrophins were used in the PR group (2318 ± 780 vs 2023 ± 530; p = 0.0211). Estradiol concentration on day of hCG (388 ± 176 vs 1022 ± 695; p = 0.0020) and oocyte yield (56.3% vs 71.6%; p < 0.0001) were significantly lower in PR group. Similarly, a trend towards a difference between the groups was found when comparing MII oocyte rate (66.5% vs 74.1%; p = 0.056). In addition, PR group showed a higher percentage of oocytes showing large periviteline space (31.8% vs 21.5%; p = 0.0202). Despite the fact that no differences were found concerning the rate of high quality embryos (49.7% vs 50.3%; p = 0.9135), pregnancy rate was significantly higher in

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FC 18-5 ART biology
Measuring the oocyte cytoplasmic maturation process with a white light optical microsystem

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Introduction Oocyte cytoplasmic maturity is difficult to assess by morphological examination under a light microscope. In ICSI, oocyte maturity is based on meiotic status and only MII are injected. In this study, cytoplasm maturity was analyzed using a microsystem recording of white light transmission spectra of the oocytes.

Materials and Methods The microsystem used to measure the absorption spectra of oocytes consisted of an optical sensor integrated into a silicon anodic bonded on to a glass slide. Two aligned optical fibers were connected to the microsystem. A white light source was launched into the illumination fiber and directed to a micro-spectrometer.

Group 1 consisted of oocytes excluded from ICSI attempts at D0 because of their maturation stage (GV or MI) or in cases of lack of spermatozoon (MII). Some MII oocytes were also issued from follicular reduction. Immature oocytes (GV and MI) were cultured to enable them to undergo final in vitro maturation and re-analysis (D0 or D1). Group 2 consisted of injected oocytes exhibiting fertilization abnormalities after ICSI at D1, with the presence of 3 pronuclei (3PN). Group 3 consisted of primordial follicles isolated from ovarian tissue. Some oocytes at different stages were fixed and embedded for microscopic examination on semithin sections or by transmission electron microscopy (TEM).

Results The 3 maturation stages (GV, MI, MII) are well identified by the progression of their transmission spectrum with their degree of maturity, but these 3 stages overlap. Spectra of 3PN without division at D2 are comparable to those of MII. Spectra of 3PN with division at D2 and spectra of primordial follicles are drastically different. TEM showed that the evolution of the spectra is in agreement with the migration of the cortical granules towards the cortex and the reduction of vesicles and vacuoles from the center of the ooplasm. In GV with abnormal spectrum, a vacuolated center of the ooplasm is observed. In MI with a spectrum of MII, cortical granules are translocated.

Conclusions Transmission spectrum analysis of oocytes with a white light optical microsystem is a minimally invasive technology allowing cytoplasm maturity to be assessed during ICSI/IVF procedures. Oocytes should not be qualified only in terms of GV, MI and MII, but also regarding their temporal evolution over these maturation stages.

FC 18-6 Economic issues
Infertility prevalence and risk factors in Vietnam

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Introduction The first research for prevalence of infertility and its risk factors in Vietnam.

Objectives To define the prevalence of infertility and its risk factors in Vietnam.

Methodology Sampling by randomized clusters in 8 representative provinces of the 8 ecological areas (60 clusters for each province). In each cluster, 30 married couples in reproductive age were interviewed with structured questionnaires standardized by WHO criteria. Logistic regression was applied to determine the relationship between risk factors and infertile couples.

Results About 14,400 couples were interviewed. The general infertility prevalence is 7.7% including primary: 3.9% and secondary: 3.8%. The highest is 13.9% in Khanh Hoa (South Central coast region), and the lowest is 5.7% in Quang Ninh (North east region). Women exposed with pesticides, alcohol drunk, abortions, delivery complications, IUD used, cervicitis are frequently more significantly infertilized than the others.

Conclusions Infertility is common reproductive health and public health issues that are considered for policy and planning making in Vietnam.

FC 18-7 ART biology
Altered global gene expressions of human placenta subjected to assisted reproductive technology treatments

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Aims Researchers are more and more concerned about the safety of fetus or offspring derived from assisted reproductive technology (ART) treatment. As the placenta is a critical organ that sustains and protects the fetus, we hypothesize that altered global gene expression of the placenta subjected to ART manipulation may reflect changes associated with ART procedures and subsequently causal related to offspring health.

Methods 3 term placenta samples were obtained from patients undergone in vitro fertilization and embryo transfer due to oviductal factors only. Other three control placentae were from those underwent normal pregnancy. A GeneChip Affymetrix HG-U133 Plus 2.0 Array was utilized to analyze the genes. Using qRT-PCR we certified microarray data from 10 dysregulated genes. Five genes were localized precisely in the placenta as per immunohistochemistry.

Results 26 differentially expressed genes were identified in the ART-treated placenta: 17 upregulated; 9 down-regulated. Eighteen of these were classified into six groups according to critical placental function: immune response; transmembrane transport; metabolism; oxidative stress; cell differentiation; and other functions. Genes involved in immune response, such as ERAP2 and ST4A, and those regulating cell differentiation, such as MUC1, were discerned to be differentially expressed. These gene products were expressed in the placental villus tissues, either in the cytoplasm or in the membrane of sncytiotrophoblastic cells.

Conclusions To our knowledge, this is the first study in comparing differentially expressed genes in placenta from patients undergone ART treatment vs. those underwent normal pregnancy. Abnormal profiles of critical placental functioning genes, such as ERAP2, ST4A and MUC1, may be valuable biomarkers to understand how the placenta affects fetal development and ART-derived offspring’s health problems.

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FC 19: Endometriosis & fertility preservation

FC 19-1 Fertility preservation
A new dynamic culture system for fresh and frozen murine ovarian tissues

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Background
Reintroduction of malignant cells by ovarian tissue autotransplantation in cancer patients seeking fertility preservation is still a potential risk. Therefore, ovarian tissue cryopreservation followed by in vitro maturation (IVM) and fertilization of oocytes could be a safer option.

Objective
As a first step to this end, the objective of this study was to establish a new dynamic culture system for fresh and frozen murine ovarian tissues.

Materials and Methods
72 ovaries were obtained from 36 female C57BL/6 mice 7-14 weeks old. 18 ovaries were frozen/thawed via slow freezing/rapid thawing protocol. 27 dynamic culture chambers (DCCs) were compared to 21 static culture chambers (SCCs), serving as a control. Each chamber contained 3 ovarian halves, which were cultured in 2 ml blank G-MOPS medium (Vitrolife, Sweden) for 6 days (1 estrus cycle) in a 38°C water bath. DCCs were continuously perfused via a peristaltic pump (Vitrolife, Sweden) for 6 days (1 estrus cycle) in a 38°C water bath. DCCs were continuously perfused via a peristaltic pump (Vitrolife, Switzerland). Estradiol (E2) and Progesterone (P) were measured daily in all effluents to assess folliculogenesis. After 6 days of culture, ovarian tissue was examined histologically for viability and follicular development, and oocytes were isolated for further IVF.

Results
DCCs with fresh tissues (n = 21) showed combined E2 and P peaks in 81% of chambers. Histologically on Day 6: viability was > 60% in 50% of chambers. Histologically on Day 6: viability was > 60% in 50% of chambers. Histologically on Day 6: viability was > 60% in 50% of chambers. Histologically on Day 6: viability was > 60% in 50% of chambers.

Conclusions
Normality of the meiotic spindle in mature MI oocytes in IVF/ICSI cycles is associated with higher fertilization rates, BL formation, numbers of usable embryos and implantation rates. All pregnancies in this study resulted from NS oocytes. In view of these findings, spindle assessment with polarised light microscopy provides an early predictor of the pregnancy potential of that oocyte. Good quality BL can be formed from AS oocytes, but these appear to have no chance of ongoing pregnancy. Thus, spindle assessment should improve the selection of the best BL for SET.
Discussion For the first time, we describe the development of frozen-thawed primate follicles from prepubertal ovarian tissue in immuno-deficient hosts up to secondary and antral stages.

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FC 19-3 Fertility preservation Melatonin inhibits apoptosis in follicles of adult mouse ovary induced by nicotine Rhaiehrahimi S.1, Mohammadghasemi F.2, Faghani M.3
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Introduction Nicotine exposure causes impaired fertility and ovarian dysfunction. The pineal hormone melatonin is known as an antioxidant agent. The aim of this study was to investigate the possible protective role of melatonin on altered ovary with nicotine.

Methods Female adult NMRI mice were divided into four groups. The control group received vehicle, group 2 received nicotine (0.4 mg/100g body weight) for 15 days, group 3 was administered melatonin (10 mg/kg) for 5 days. Group 4 received both nicotine (0.4 mg/100g body weight) and melatonin (10 mg/kg). All animals were treated intraperitoneally. After autopsy on 16th day, evaluations were made by histopathology and in situ TUNEL assay. Statistical analysis was performed using ANOVA test.

Results Nicotine significantly reduced the number and size of pre-antral and antral follicles compared to the control (p < 0.01). However the numbers of primordial follicles in nicotine-treated group were reduced but it was not significant. A significant increase in atretic follicles were observed in nicotine-treated animals compared to the control (p < 0.01). Ovaries of mice exposed to nicotine had a significant increase in the percentage of apoptotic cells compared to controls (11.8 ± 0.03 vs 3.6 ± 0.01), while melatonin in group 4 caused a marked normalization in number and size of ovarian follicles compared to group 2 (p < 0.01). The percentage of apoptotic cells were significantly reduced in group 4 compared to the group 2 (6.2 ± 0.01 vs 11.8 ± 0.03).

Conclusions The results from this study suggest that melatonin may have a protective effect against nicotine induced ovarian damage through reduction of apoptosis and probably by decreasing oxidative stresses.

Funding Support for this study was provided by GUMs.

FC 19-4 Endometriosis IL-17 stimulates migration of neutrophils through endometriotic stromal cell-derived GROα

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Endometriosis, defined by the presence of viable endometriotic tissue outside the uterus, is an enigmatic disease. Numerous lines of evidence suggest that aberrant immune responses and inflammatory reactions are involved in the pathogenesis of endometriosis. Th17 cells, which secrete their specific cytokine IL-17, are a newly discovered helper T cell subset, and suggested to be associated with pathology of many autoimmune diseases. Th17 cells are present in endometriotic tissues and IL-17 stimulates IL-8 secretion from endometriotic stromal cells (ESCs). However, the function of IL-17 in endometriosis is not fully understood. To investigate the effect of IL-17 on endometriosis, we cultured ESCs obtained from patients with ovarian endometriomas undergoing laparoscopy after obtaining informed consent. We stimulated ESCs with IL-17 100ng/ml or vehicle for 6h, and examined the expression of 24413 genes using microarray system. We detected GROα as the most up-regulated gene (23.6 fold), so we further studied the effect of IL-17 on GROα produc- tion using ELISA. IL-17 enhanced GROα secretion from ESCs in a dose-dependent manner. As GROα is known to function as a neutrophil chemotactic factor, we studied the effect of the conditioned medium of IL-17-stimulated ESCs on neutrophil chemotaxis using Boyden chamber method. The conditioned medium attracted more neutrophils than that of non-stimulated ESCs (2.1 fold; p < 0.05). This effect was partly inhibited by pretreatment with anti-GROα neutralizing antibody, compared to control isotype IgG (0.59 fold; p < 0.05). We also conducted immunohistochemistry of GROα, IL-17, and neutrophil elastase. As expected, cells positive for these proteins are colocalized in the same stromal area beneath the epithelium. Collectively, it is speculated that IL-17 attracts neutrophils by upregulating GROα production from stromal cells in endometriotic tissues. In view of our previous finding that proteases from neutrophils enhance the production of inflammatory cytokines and the proliferation of ESCs through protease activated-receptor 2, IL-17 may stimulate the progression of endometriosis by enhancing the production of GROα from ESCs and result- ing neutrophil recruitment to the lesion. This works was partially supported by Grants-in-Aid for scientific research from Ministry of Health, Labor, and Welfare.

FC 19-5 Endometriosis Association between heavy metals and endometriosis: Results of a preliminary study Silva N.V.1, Sananayaka H.2, Periris John R.2, Wickremasinghe R.1, Waduge K.1
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Objective To determine the association between exposure to heavy metals and heavy metal concentrations in whole blood and ectopic endometrial tissue in endometriosis.

Design, Setting and Methods A case-control study was conducted in a tertiary care hospital. Women with endometriosis confirmed by either laparoscopy or laparotomy (n = 118) were compared with age-matched controls with no evidence of endometriosis at laparoscopy or laparotomy (n = 109). Information on exposure to environmental pollution was collected using a pre-tested interviewer administered questionnaire after obtaining informed written consent. In a sub group of cases (n = 14) and age matched controls (n = 11) whole blood samples were collected. In 10 cases ectopic endometrial tissue samples were obtained by a single surgeon. After measuring the wet weight, whole blood samples and ectopic endometrial tissues were digested with supra pure 65% HNO3 and analyzed for heavy metals by the Total Reflection X-ray Fluorescence (TXRF) technique. The association between exposure to environmental pollution and endometriosis was determined by chi square tests and odds ratios(OR) using SPSS. Means of the log transformed metal levels were compared using t-tests.

Results Endometriosis was commor in those living in close proximity to a main road (OR = 4.195; 95 % CI: 2.309–7.621). In multivariate analysis, the risk of endometriosis was 2.93 (p = 0.02) times greater among those living in close proximity to a main road as compared to those who were not living in close proximity to a main road after adjustment. Mean (+SD) blood levels of nickel in the cases (2.19 ± 0.45 PPB) was significantly higher than in the controls (0.65 ± 0.14 PPB; p = 0.029). The mean (+SD) lead levels in the cases (21.85 ± 1.99 PPB) and controls (17.85 ± 1.5 PPB) were similar (p = 0.378). Tissue samples had higher mean (+SD) levels of nickel (9.3 ± 15.31 PPB) and lead (66.60 ± 10.37 PPB) as compared to blood levels of nickel (0.502 ± 0.406 PPB) and lead (18.16 ± 1.58 PPB) which were statistically significant (p = 0.003 and p = 0.025 respectively).

Conclusions Endometriosis may be an environmentally induced disease probably due to exposure to exhaust fumes from motor vehicles. Patients with endometriosis had higher levels of nickel and there is evidence that nickel accumulates in endometrial tis-
FC 19-6 Endometriosis
Simvastatin reduces endometrial stromal cell invasiveness

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Objectives Recently we have reported that statins, the competitive inhibitors of the key enzyme regulating the mevalonate pathway, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, decrease proliferation of human endometrial stromal (HES) cells. Furthermore, we found that simvastatin treatment reduces the number and the size of endometriotic implants in a nude mouse model of endometriosis. The present study was undertaken to investigate the effect of simvastatin on invasiveness of HES. In addition, since actions of statins may be due to reduced prenylation, the study investigated whether invasiveness of HES cells was affected by prenylation substrates: farnesyl-pyrophosphate (FPP) and geranylgeranyl-pyrophosphate (GGPP).

Methods HES cells were isolated from endometrial biopsies (n = 6). 24-well plates with transwell inserts containing polycarbonate membranes with 8.0 µm pores were coated using Matrigel. The cells were suspended in phenol red-free, serum-free DMEM with 1mM estradiol and placed on the transwell inserts. The lower chambers of the wells were filled with phenol red-free DMEM with 1mM estradiol and 10% Charcoal/Dextran treated FBS used as a source of cholesterolactants. Subsequently, the cells were cultured for 24 hrs without (control) or with simvastatin (10–30 µM) and/or GGPP (30 µM) and FPP (30 µM). Then, non-invading cells were scraped from the top of the transwell inserts while invading cells were fixed, stained and counted under a light microscope. The mean number of cells was calculated from four replicates per experiment and expressed as a percentage of control. To evaluate possible toxicity of simvastatin, GGPP and/or FPP on the HES cells, the effect of treatment on the number of viable cells was evaluated in parallel experiments by MTS assay.

Results Simvastatin (30 µM) reduced HES cell invasiveness by 62% (p < 0.0001). GGPP and FPP alone, did not affect HES invasiveness, however the inhibitory effect of simvastatin was abrogated by GGPP, but not by FPP. In all experiments, 24-hour exposure to simvastatin, GGPP and FPP had no significant effect on the number of viable cells.

Conclusions Simvastatin inhibits HES cell invasiveness. Reversal of simvastatin effects by GGPP indicates that the inhibitory effect of simvastatin is, at least in part, related to reduction of geranylgeranylation. The present findings provide a novel explanation for the beneficial effects of simvastatin on endometriosis.

FC 19-7 Fertility preservation
Lifestyle factors and eating habits influence on intracytoplasmic sperm injection cycles outcomes

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Introduction Information about influence of habits on assisted reproduction techniques (ART) is not plentiful. This study aimed to investigate the relationship between specific social and feeding habits and intracytoplasmic sperm injection (ICSI) cycles outcomes.

Materials and Methods Questionnaires about social and feeding habits, obtained from 126 women undergoing ICSI were evaluated. The patients were asked about (i) physical activity practice, (ii) smoking habits and the consumption of (iii) whole bread, (iv) whole grains, (v) fruit, (vi) milk and dairy products, (vii) vegetable, (viii) caffeine-containing soft drinks, (ix) coffee, (x) alcohol and (xi) chocolate bars. The results were analyzed by logistic regression and Pearson correlation models. The results were expressed as odds ratios (OR), 95% confidence intervals (CI), or regression coefficient (RC) and p-values.

Results The logistic regression model, demonstrated a nearly 4-fold increase in the pregnancy rate in patients which reported whole bread intake (OR: 3.80; CI: 1.00–14.48; p = 0.05). On the other hand, chocolate bars consumption (OR: 0.26; CI: 0.08–0.85; p = 0.026) and soft drinks consumption (OR: 0.38, CI: 0.09–0.79; p = 0.035) were found to decrease the odds of pregnancy. Fertilization rate was increased in patients who consume vegetables (RC: 20.11; p = 0.021). Physical activities significantly improved implantation rate (RC: 25.8; p = 0.05) and smoking habit was correlated with higher chance of miscarriage (RC: 20.0; p = 0.067).

Conclusions Although our results showed that physical activities, whole bread and vegetables intake may improve ART outcomes, we suggest that these specific habits themselves may not be the reason for the improved outcomes, but rather a combination of factors related to a healthy lifestyle. Indeed, we also observed that unhealthy habits such as smoking and consumption of chocolate and soft drinks impaired the reproductive outcomes. It is known that specific habits directly or indirectly affect ovarian function, folliculogenesis, growth, implantation and development of a clinical pregnancy. However, since different habits may be expressed by the same person, the identification of a single habit which may affect ART outcomes is difficult. Therefore, before ART begins, patients should be advised about the positive effect of the combination of healthy habits on the reproductive outcomes.

Funding none.

FC 19-8 Endometriosis
Immortalized cell lines are suitable for studying endometriosis

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Introduction For studying endometriosis in vitro, we used several immortalized stromal and epithelial cell lines isolated from endometrial and endometriotic tissues.

Every biological function analyzed was compared with typical characteristics of endometriosis. Especially transforming growth factor (TGF)-beta and beta2 were used as markers, because TGF-beta-2 are increased during menstruation and endometriosis and were described to modulate effects of progesterone on endometrial or endometriotic cells, primarily on secretion of matrix metalloproteinases (MMPs).

Methods We used 2 stromal and one epithelial cell line from normal human endometrium and one stromal and three epithelial cell lines from endometriotic patients. Cell proliferation was measured directly by counting the cells with a CasyCounter. Protein secretion was quantitated with ELISAs and apoptosis determined with FACS analysis.

Results All cell lines secreted TGF-beta1, but remarkably the endometriotic cell lines secreted considerably higher levels compared to normal endometrial cells. Of note, only the endometriotic cells secreted TGF-beta2. However, all cell lines only produced very low levels of TGF-beta3. All cell lines could be stimulated by TGF-betas because they express the high-affinity receptors TGF-beta receptor type 1 (TBR1) and TBR2. Treatment with TGF-betas reduced cell proliferation by inducible apoptosis at high cell numbers but induced proliferation at low cell numbers. In contrast, TGF-beta3 reduced cell proliferation only moderately. Pregnancy activator inhibitor-1 (PAI-1) responds strongly to stimulation with TGF-betas. We observed a strong secretion of PAI-1 in most cell lines but a consistently higher secretion by endometriotic cells compared to endometrial cells. TGF-beta3 increased secretion of PAI-1 tremendously. Progesterone reduced cell proliferation of epithelial endometriotic...
cells dose-dependently and reduced secretion of MMP2. In contrast, progesterone-induced secretion of TGF-beta1 and TGF-beta2.

Conclusions The results obtained with anastomiotial and endometriotic cell lines are highly consistent with published data. For example TGF-beta1 is the predominant TGF-beta isoform in explant cultures as well as in endometriosis, followed by TGF-beta2 and very low levels of TGF-beta3. Similarly PAI-1 levels were found to be elevated in endometriosis. Our results convincingly demonstrate that immortalized cell lines are a suitable model for studying endometriosis.

FC 20: Male infertility & fertilization

FC 20-1 Male infertility

Chromatin integrity in human ejaculate spermatozoa of smokers and non-smokers patients and its relationship to seminal oxidative stress parameters

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Aims The purpose of present study was to evaluate the consequences of cigarette smoking on the sperm chromatin integrity of smokers and non-smokers patients.

Methods Ejaculates from 116 patients of couples consulting for infertility were divided into 2 groups: (i) smokers (n = 53) and (ii) non-smokers (n = 63). Sperm chromatin integrity was evaluated using Chromomycin (CMA) and TUNEL assay methods. The smoking metabolite (Cotinine) was evaluated in seminal plasma by Enzyme Linked Immunosorbant Assay (ELISA) technique and the levels of Reactive Oxygen Species (ROS) and 8-Hydroxyguanosine (8-OHdG) were assessed by ELISA assay, whereas Malondialdehyde (MDA) levels assessed by chemical reactions.

Results Both parameters CMA, and TUNEL were significantly higher (p < 0.01) in smokers (36.4 ± 8.1%, 17.4 ± 5.3%) in comparison to non-smokers (29.8 ± 7.1%, and 11.3 ± 4.2 respectively).

Sperm DNA fragmentation and non-condensed chromatin were significantly negatively correlated with the conventional parameters. The ROS (umol/l), MDA (µM), 8-OHdG (ng/ml) and cotinine (ng/ml) concentrations were significantly higher (p < 0.010) in smokers (138.5 ± 41.9, 9.0 ± 1.46, 3.0 ± 1.8, 99.6 ± 64.6) than that of non-smokers (52.7 ± 21.8, 1.1 ± 0.8, 0.6, and 2.7 ± 2.4, respectively). Likewise, these oxidative stress biomarkers were inversely correlated with the conventional parameters.

Conclusions These findings suggest that smoking may be a cause of oxidative stress elevation and consequently deteriorate semen quality especially sperm nuclear integrity which disrupting sperm functions.

FC 20-2 Male infertility

Declining quality on semen collected for ICSI cycle when compared to previous semen analysis collected for infertility evaluation

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Introduction: Semen analysis is the cornerstone evaluation of the fertile man. In order to better evaluate and treat the infertile couple, a good semen analysis evaluation is an important step. It is well described that semen quality decreases when the man is under pressure. Also, it has been suggested that participation in in vitro fertilization (IVF) programs imposes certain level of stress in some men, which may be associated with a reduction in semen quality. The aim of this study was to investigate possible differences in semen quality when samples were collected to be used in intracytoplasmic sperm injection (ICSI) cycles and samples collected for simple infertility evaluation purposes.

Materials and Methods: In this retrospective clinical study, semen samples were obtained from 617 male partners of women submitted for ICSI cycles between January 2006 and December 2009. All samples were collected in the same room and evaluated by the same person. Semen specimens were collected after 3–5 days of abstinence and were analyzed for sperm concentration and percent progressive motility according to World Health Organization criteria and sperm morphology according to Tygerberg’s strict criteria. Differences in semen parameters between sperm samples collected for instant use in ICSI cycles compared to previous sperm analysis in the same man were analyzed statistically.

Results: Sperm concentration was significantly lower (p = 0.03) in semen samples collected on the day of oocyte retrieval (17.2 ± 5.02) compared to those samples provided for previous semen analysis for infertility evaluation (29.2 ± 9.1). Also, sperm motility decreased from the day of infertility investigation (52.7 ± 21.8) to the day of oocyte retrieval (31.7 ± 12.9; p = 0.04). On the other hand, no differences were found on sperm morphology according to the Tygerberg’s strict criteria between the day of infertility investigation (5.1 ± 1.8) and the day of oocyte retrieval (4.3 ± 2.7; p = 0.08).

Conclusions: Our results suggest that the decrease in semen quality is at least partly result of the acute psychological stress that is experienced by the patients who are requested to provide a semen sample at the day of in vitro fertilization. Due to this decrease in semen quality, semen cryopreservation may be suggested for men with severe oligospermia to overcome azoospermia/severe oligospermia on the day of ICSI procedure.

Funding none.

FC 20-3 Male infertility

Effect of varicocelectomy on semen parameters, sperm DNA and plasma membrane integrity

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Introduction: Varicocele has been associated with deleterious effects on semen parameters, sperm DNA and sperm membrane integrity.

Purpose: To evaluate the effect of varicocelectomy on semen parameters, levels of sperm DNA damage and sperm membrane integrity in infertile men with varicocele.

Materials and Methods: 33 consecutive oligoasthenozoospermic patients with bilateral varicocele and 40 fertile men served as a control group were included in this study. All subjects underwent history taking, physical examinations and scrotal Doppler sonography for assessment of the presence and severity of varicocele. World Health Organization semen analysis, sperm DNA damage – expressed as the DNA fragmentation index using the acridine orange stained and viewed under fluorescence microscope – and sperm membrane integrity assessed by hypo-osmotic swelling test were assessed preoperatively and 6 months after subinguinal varicocele ligation.

Results: Mean sperm count, progressive sperm motility and abnormal sperm morphology, sperm DNA fragmentation index and hypo-osmotic swelling improved significantly after varicocelectomy from (23.17 ± 2.65, 28.33 ± 2.93, 45.45 ± 2.623, 29.03 ± 2.365, 23.39 ± 1.151) to (34.64 ± 2.97, 37.42 ± 3.609, 33.42 ± 2.380, 21.09 ± 1.450, 40.17 ± 1.529) respectively (p < 0.05 for each). In spite of postoperative significant improvement in semen parameters, DNA fragmentation index and plasma membrane integrity, they are still significantly lower than the control group (p = 0.002; p = 0.024) respectively. In the present study, sperm DNA fragmentation index showed negative significant correlation with progressive sperm motility (r = 0.391; p < 0.0001), plasma membrane integrity (r = 0.301; p = 0.002) and positive significant correlation with abnormal sperm morphology (r = 0.264; p = 0.006). In addition, the results of the hypo-osmotic swelling test correlated positively with sperm count, abnormal sperm morphology and cor-
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Introduction
Deletions of azoospermic factor regions (AZFAs, b and c) of the Y chromosome are well characterized to connect with male spermatogenesis. Conversely, partial deletions of AZFc region are still controversial issues showing that phenotypic symptoms of these deletions are inconsistent to male haplogroups. In this study, we analyze deletions of Y chromosome and observe aspects of infertility according to Y chromosome deletions in Japanese population.

Materials and Methods
We studied 1254 infertile and 374 fertile men. Infertile group were divided as 518 men with azoospermia, 261 men with severe oligozoospermia (≤ 5×10⁶/ml), 173 men with oligozoospermia (5×10⁶/ml ≤ > 20×10⁶/ml) and 302 men with normozoospermia. 374 fertile men used as control with normozoospermia. To find deletion discrepancies, we screened throughout SRTY on Yp to y1201 on Yq using STS approach. Based on 8 markers, subjects were partitioned into D*(xD2), D2, E, F* (xK), K*(xO), N and O.

Results and Discussion
In total, 1628 subjects are analyzed for our study. Major three criteria are only found in infertile group. Especially, AZFA (6/518) and AZFB (8/518) deletions are exclusively existed in azoospermia whereas AZFc deletion is distributed in azoospermia (3/518) and severe oligozoospermia (10/261) indicating that these deletions are owing to typical phenotypic abnormality. Also we focus on partial deletions of AZFc region. The most abundant deletion type was gr/gr deletion which was occurred 36.2% (590/1628) frequencies and evenly distributed among infertile and control group. Approximately 90% of gr/gr deleted subjects were bounded for haplogroup D lineage. In case of b2/b3 deletion, the frequency of b2/b3 deletion (27 cases) ranked on the second but the frequency of infertile (1.8%, 24/1254) and control group (0.8%, 3/374) was drastically different suggesting that b2/b3 deletion seems to high risk factor of infertility. b1/b3 deletion is found in only 7 cases in total but there is no significant difference between infertile and control group. Considering with haplogroup, haplogroup D is highly restricted to occur b1/b3 and b2/b3 deletion which display that haplogroup D escape from high risk factor of deletions owing to gr/gr deletion.

Conclusions
We concluded from this study that semen parameters, sperm DNA and plasma membrane integrity are impaired in patients with varicocele and these parameters improved significantly after varicocele ligation. However, they failed to reach their values in fertile patients.

FC 20-4 Male infertility
Partial and complete deletions of AZF regions of Y chromosome in Japanese male population
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Introduction
Deletions of azoospermic factor regions (AZFAs, b and c) of the Y chromosome are well characterized to connect with male spermatogenesis. Conversely, partial deletions of AZFc region are still controversial issues showing that phenotypic symptoms of these deletions are inconsistent to male haplogroups. In this study, we analyze deletions of Y chromosome and observe aspects of infertility according to Y chromosome deletions in Japanese population.

Materials and Methods
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Results and Discussion
In total, 1628 subjects are analyzed for our study. Major three criteria are only found in infertile group. Especially, AZFA (6/518) and AZFB (8/518) deletions are exclusively existed in azoospermia whereas AZFc deletion is distributed in azoospermia (3/518) and severe oligozoospermia (10/261) indicating that these deletions are owing to typical phenotypic abnormality. Also we focus on partial deletions of AZFc region. The most abundant deletion type was gr/gr deletion which was occurred 36.2% (590/1628) frequencies and evenly distributed among infertile and control group. Approximately 90% of gr/gr deleted subjects were bounded for haplogroup D lineage. In case of b2/b3 deletion, the frequency of b2/b3 deletion (27 cases) ranked on the second but the frequency of infertile (1.8%, 24/1254) and control group (0.8%, 3/374) was drastically different suggesting that b2/b3 deletion seems to high risk factor of infertility. b1/b3 deletion is found in only 7 cases in total but there is no significant difference between infertile and control group. Considering with haplogroup, haplogroup D is highly restricted to occur b1/b3 and b2/b3 deletion which display that haplogroup D escape from high risk factor of deletions owing to gr/gr deletion.

Conclusions
We concluded from this study that semen parameters, sperm DNA and plasma membrane integrity are impaired in patients with varicocele and these parameters improved significantly after varicocele ligation. However, they failed to reach their values in fertile patients.

FC 20-5 Male infertility
Vitrification sperm bank: The new aspetic technique for human spermatozoon cryopreservation
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Introduction
Nowadays, the conventional freezing of spermatozoa is used for the preservation of fertility in patients with cancer or for programs in reproductive medicine. However, these techniques causes notorious chemical-physical damage to the extracellular and intracellular sperm membranes, due to an increased lipid peroxidation, decrease of the sperm motility, mitochondrial activities, and induction apoptosis markers. As an alternative, we established an aspetic technique that preserves just the sperm cells without seminal plasma and permeable cryopreservants.

Materials and Methods
The sperm suspension obtained after swim-up is centrifugated and separated to conventional freezing or vitrification technique. The sperm suspension (0.25 M sucrose and HTF-HSA 1%) is placed in 0.25 ml insemination straw (IE). The warming is performed by quick direct submerging of O.25 IE, into 5ml of HTF-HSA 1% (pre-warmed to 37º C). The testicular weight of the WT-*EAO* mice was significantly decreased after the first immunization mice were sacrificed, testes were weighted and either fixed in Bouin’s solution or homogenized for cyto-kine evaluation. A scoring system for EAO was developed based on histology examination. The disease progression was sub-divided into five stages and the animals were grouped accordingly. IL-6, TNFα and INFγ levels in the testes and spleens were measured using specific ELISA.

Results
The testicular weight of the WT-EAO-mice was significantly decreased after 50 days compared to the control group (p < 0.001). Testes showed severe morphological changes, including germ cell sloughing, disruption of spermatogenesis, Sertoli-cell-only syndrome and massive interstitial cell infiltration. ELISA measurements revealed higher levels of IL-6, TNFα and INFγ in testes of EAO-mice starting gradually from day 30 compared to adjuvant controls. At day 70 differences between EAO and adjuvant group were not distinguishable (3-fold elevated levels of the respective cytokines. Cytokine levels tended to coincide with the

Conclusions
These results suggest the possibility for eliminating the use of liquid nitrogen to freeze the male gamete. This vitrification technique, that allows to preserve most the functions of sperm cells, is an easy, speedy and cheap alternative for sperm bank cryopreservation. On the other hand, as the technique uses spermatozoon free of seminal plasma components, it can be applied to samples with different pathologies (AIDS and other sexual transmission diseases).

FC 20-6 Male infertility
Involvement of IL-6, TNFα and INFγ in the pathogenesis of experimental autoimmune orchitis; distinct expression in the testes and spleen
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Introduction
Orchitis is characterized by inflammatory infiltrates in the testicular interstitium and is associated with focal or total impairment of spermatogenesis. Cytokines are expressed at basal levels in the testis and are considered to play a major role in the maintenance of normal testicular function.

 Aim
The aim of this study is to investigate the expression levels of IL-6, TNFα and INFγ in testes and spleen during the progression of experimental autoimmune orchitis (EAO) in mice.

Materials and Methods
Induction of EAO: Testes of adult wild type (WT) BALB/c mice were homogenized, mixed with complete Freund’s adjuvant and injected subcutaneously in tail, neck and foot pad of Balb/c mice. Pertussis toxin was used as a co-adjuvant. In adjuvant control groups testis homogenate was replaced with saline. At day 0 (untreated group), 20, 30, 40, 50 and 70 after the first immunization mice were sacrificed, testes were weighted and either fixed in Bouin’s solution or homogenized for cytokine evaluation. A scoring system for EAO was developed based on histology examination. The disease progression was subdivided into five stages and the animals were grouped accordingly. IL-6, TNFα and INFγ levels in the testes and spleens were measured using specific ELISA.

Results
The testicular weight of the WT-EAO-mice was significantly decreased after 50 days compared to the control group (p < 0.001). Testes showed severe morphological changes, including germ cell sloughing, disruption of spermatogenesis, Sertoli-cell-only syndrome and massive interstitial cell infiltration. ELISA measurements revealed higher levels of IL-6, TNFα and INFγ in testes of EAO-mice starting gradually from day 30 compared to adjuvant controls. At day 70 differences between EAO and adjuvant group were not distinguishable (3-fold elevated levels of the respective cytokines. Cytokine levels tended to coincide with the
severity of diseases as assessed by the histoscore. On average, basal testicular levels (untreated group) of IL-6, TNFα and IFNγ were 2–3 fold higher than in spleen (Surprisingly, in EAO animals splenic concentrations were decreased 2–3 fold compared to adjuvant control group, which remained at basal levels.

**Conclusions** IL-6, TNFα and IFNγ are completely differently regulated in testes and spleen after induction of EAO. Thus, local testicular cytokine production seems to play a much more relevant role in the pathogenesis of EAO than their systemic contribution.

**Diemer T.1, Weidner W.1, Steger K.1**

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**FC 20-7 Male infertility Evaluation of testicular biopsies from infertile men: Correlations between histopathology, sperm retrieval, and protamine mRNA expression**

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**Objectives** In patients with azoospermia, detailed clinical work-up does not allow predicting definitively the presence of mature elongated spermatids in the testis. Hence, histological evaluation of testicular biopsies remains a key investigation in reproductive medicine and important predictor of successful testicular sperm extraction (TESE). Moreover, the outcome of assisted fertilisation after TESE has been related to the expression of protamines (Prm) in testis. The significance of Prm contents in paraffin-embedded sections of fixed testicular biopsies has to be elucidated in further analyses.

**Materials and Methods** Bilateral testicular biopsies were obtained from 43 azoospermic patients (aged 23–51 years) in 2008 by a multi-focal approach (3 localizations per testis). For histopathological evaluation, tissue specimens were fixed in Bouin’s solution, embedded in paraffin, and processed according to routine protocols. Semi-quantitative assessment of spermatogenesis was based on a score count calculating the percentage of tubules revealing elongated spermatids within a given slide. After RNA extraction from deparaffinized tissue sections and cDNA synthesis, degenerated oligonucleotide primed-PCR was performed, followed by quantitative real-time PCR with primers for Prm1 and Prm2. For “diagnostic” sperm retrieval (TESE trial), tissue specimens cryopreserved under “therapeutic” conditions were used.

**Results** Score count evaluation of spermatogenesis closely correlated with testicular volume, serum FSH levels, and results of a TESE trial (p < 0.01 for each parameter, resp.). In 18 of 43 patients, score counts revealed significant differences between contralateral tests and within the same organ. Similarly, Prm1/2-mRNA contents varied markedly among the tissue specimens obtained from multi-focal incisions, with Prm1 contents exceeding those of Prm2 in all samples tested. A significant correlation between Prm mRNA levels detected in fixed testicular tissue and histological evaluation was found only in selected localizations/patients.

**Conclusions** Our results confirm the high prognostic value of a score count evaluation of spermatogenesis with regard to successful TESE. The significance of Prm contents in paraffin-embedded sections of fixed testicular biopsies has to be elucidated in further analyses.

**FC 20-8 Male infertility Prevalence of poor semen parameters in men with metabolic syndrome**

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**Objectives** To determine the prevalence of poor semen parameters in men with metabolic syndrome coming for fertility evaluation.

**Setting** Teaching hospital.

**Methods** All men attending the fertility clinic from August 2008 to March 2009 were screened for metabolic syndrome. Evaluation of anthropometric variables, blood pressure, fasting blood glucose and lipids was performed. Subjects (624) were examined, fasting blood samples were available in 526 men and analyzed for prevalence of metabolic syndrome. Metabolic syndrome was diagnosed using the United States Adult Treatment Panel-3 (ATP-3) guidelines.

**Results** Metabolic syndrome was present in 159 (30.22%) subjects. The prevalence in the general population in the same city has been reported as 18.3% (p < 0.001). Sperm concentration below 15 million/ml was seen in 74 men (46.5%) men with metabolic syndrome and in 181 (49.4%) men without the same. Similarly those with motility less than the WHO standards were 40 (25.2%) and 106 (28.8%) in the 2 groups respectively. Both these parameters were not statistically significant between the 2 groups.

**Conclusions** There is a higher prevalence of metabolic syndrome in men coming for fertility treatment compared to the general population. However the semen parameters are not significantly different between those with or without metabolic syndrome. Larger studies are needed to look for a causal relationship and the role of lifestyle changes in such men in improving their sperm parameters.

**FC 21 Male infertility & fertilization II**

**FC 21-1 Male infertility Differential relationships between P- and G-pattern, normal sperm morphology and DNA fragmentation**

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**Introduction** Normal sperm morphology has been found to be predictive of fertilization in vitro. Another parameter, DNA integrity, has been shown to be positively correlated with pregnancy but not fertilization. In this study, the rate of sperm DNA fragmentation in the different normal sperm morphology groups was investigated.

**Materials and Methods** In a total of 79 men sperm concentration, motility, normal sperm morphology according to strict criteria and sperm DNA fragmentation was determined by means of a flowcytometric TUNEL assay where the following parameters were detected and calculated, respectively: % TUNEL-negative sperm (TUNEL-neg. %), mean channel fluorescence of TUNEL-negative sperm (TUNEL-neg. MCF), relative fluorescent activity of TUNEL-negative sperm (TUNEL-neg. RA), DNA fragmentation index as percentage of the mean channel fluorescence in the TUNEL assay (TUNEL DFI MCF) and DNA fragmentation index as percentage of the relative fluorescent activity in the TUNEL assay (TUNEL DFI RA). In addition, data (n = 67) (% TUNEL-neg. sperm determined by means of fluorescence microscopy and normal sperm morphology) from a previous project were re-evaluated. In both sets of data, sperm morphology was divided into two groups, namely P-pattern (0–4% normal morphology) and G-pattern (5–14% normal morphology) and was then statistically evaluated.

**Results** Direct correlation between normal sperm morphology and the different parameters of the TUNEL assay after flowcytometry revealed positive, but not significant relationships. Only sperm concentration was significantly correlated with morphology (r = 0.317; p = 0.0051). However, if morphology was grouped into P- and G-pattern and correlated separately, significant positive correlations were found within the P-pattern group for motility, TUNEL-neg. RA, TUNEL-neg. % and TUNEL-neg. DFI RA, while none of the relationships for the G-pattern group was significant. Scatter diagrams revealed significantly different slopes between P- and G-pattern morphology. The same pattern was observed when analyzing the archived data deriving from fluorescence microscopy.

**Conclusion** Although positive correlations have been described between normal
sperrn morphology and DNA damage, these data indicate that particularly P-pattern sperrn morphology is indicative of poor DNA frag-
mamentation and thus leading to decreased im-
plantation and pregnancy rates. Results also give reason for the better prognosis of G-pattern morphology ejaculates in IVF.

**FC 21-2 Male infertility**

**Impact of oxidative stress, leukocytes and round cells on sperm parameters of infertile patients**

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**Aims** The objectives of this study were the determination of markers of oxidative stress, antioxidant capacity, chromatin condensation and structure integrity. DNA fragmentation in sperm cells and its correlation with parameters of sperrnogram, also to clarify the role of WBC, round cells and LPO in male infertility.

**Methods** Semen samples collected by masturbation from 123 infertile men were examined routinely according the WHO guidelines (1999). MDA, ROS and TAS levels in sperm cells were measured. Chromo-
mycin (CMA3) was used for assessment of chromatin condensation. Chromatin structure integrity was determined after treatment of samples with acridine orange and DNA frag-
mamentation was assessed using TUNEL assay.

**Results** A statistically significant correlation was shown between MDA and WBC (p < 0.001) and “round cells” (p = 0.0084).

**Conclusions** Correlation between MDA and leukocytes as well as “round cells” points to an association of these cells and the induc-
tion of lipid peroxidation in sperm cells and may explain the decrease of the fertilizing potential of spermatozoa in infertile patients.

**Materials and Methods** This was a pro-
spective randomized trial of 348 females aged 24–38, with different degree of peri-
adnexal adhesions due to prior pelvic inflamm-
atory disease (PID). They were investi-
gated between 2004 to 2010. Ultrasound, hysterosalphingography, computerized kymo-
perturbation and laparoscopy have been used for definition of tubo-peritoneal factor of in-
fertility.

**Result** Group 1 of 183 patients with tubal occlusion (127 hydrosalpinges and 56 isth-
mic tubal lesions) and Group 2 of 165 pa-
tients with peritoneal form of infertility were selected for the study. Analysis of the results revealed prognostic value of computed kymoperturbation by seduxen test for diag-

nosis of isthmic tubal occlusion before laparoscopy. Kymoperturbation cancelled diagnosis of isthmic tubal occlusion in 12% of patients and laparoscopy in turn confirmed the stated diagnosis, thereby emphasizing significance of kymoperturbation. In cases of tubal occlusion laparoscopic fimbrioplasty and neosalpingosyntesis carried out. The chance to regain fertility after reconstructive surgery is high in patients with mild post-

inflammatory peridnexal adhesions (26.3% of pregnancies have been revealed in Study Group 1 and 30.7% in Group 2). Reproduc-
tive function was restored in patients, whose range of basal pressure was 45 to 66 mmHg, frequency of contraction not less than 9 and form index range 26.6–34.33%.

**Conclusion** Kymoperturbation is a new, less traumatic and noninvasive pre- and post-

operative diagnostic tool compared to other types of investigations for tubal infertility. It makes available to observe and study func-
tional state of tubes in dynamics. Normalization of basal pressure, frequency of contrac-
tion and form index is the good and optimis-
tic sign of successful treatment and restora-
tion of the reproductive function in women with tubal infertility. In case of failure and undesirable outcomes of surgical correction the tactics of 6–12 months waiting is justi-
fied, because most of pregnancies have been revealed during this period. If waiting period is not successful and reproductive function has not been restored after surgery, patients are recommended to use in vitro fertilization.

**FC 21-3 Fertilization**

**State of fallopian tubes in patients after surgery for tubo-peritoneal infertility**

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**Objectives** The aim of the study was evaluation of the functional and anatomy state of fallopian tubes in patients with tubo-peritoneal infertility before and after surgery.

**Materials and Methods** This was a pro-
spective randomized trial of 348 females aged 24–38, with different degree of peri-
adnexal adhesions due to prior pelvic inflamm-
atory disease (PID). They were investi-
gated between 2004 to 2010. Ultrasound, hysterosalphingography, computerized kymo-
perturbation and laparoscopy have been used for definition of tubo-peritoneal factor of in-
fertility.

**Result** Group 1 of 183 patients with tubal occlusion (127 hydrosalpinges and 56 isth-
mic tubal lesions) and Group 2 of 165 pa-
tients with peritoneal form of infertility were selected for the study. Analysis of the results revealed prognostic value of computed kymoperturbation by seduxen test for diag-

nosis of isthmic tubal occlusion before laparoscopy. Kymoperturbation cancelled diagnosis of isthmic tubal occlusion in 12% of patients and laparoscopy in turn confirmed the stated diagnosis, thereby emphasizing significance of kymoperturbation. In cases of tubal occlusion laparoscopic fimbrioplasty and neosalpingosyntesis carried out. The chance to regain fertility after reconstructive surgery is high in patients with mild post-

inflammatory peridnexal adhesions (26.3% of pregnancies have been revealed in Study Group 1 and 30.7% in Group 2). Reproduc-
tive function was restored in patients, whose range of basal pressure was 45 to 66 mmHg, frequency of contraction not less than 9 and form index range 26.6–34.33%.

**Conclusion** Kymoperturbation is a new, less traumatic and noninvasive pre- and post-

operative diagnostic tool compared to other types of investigations for tubal infertility. It makes available to observe and study func-
tional state of tubes in dynamics. Normalization of basal pressure, frequency of contrac-
tion and form index is the good and optimis-
tic sign of successful treatment and restora-
tion of the reproductive function in women with tubal infertility. In case of failure and undesirable outcomes of surgical correction the tactics of 6–12 months waiting is justi-
fied, because most of pregnancies have been revealed during this period. If waiting period is not successful and reproductive function has not been restored after surgery, patients are recommended to use in vitro fertilization.

**FC 21-4 Male infertility**

**Estrogen-dependent signaling pathway in spermatozoa in group of patients with idiopathic sterility**

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Hidden defects in human spermatozoa dis-
turbing: capacitation, transport in the repro-
ductive female tract, process of interaction of spermatozoa and proper embryo develop-
ment may be the reasons responsible for lack of a guarantee with in the idiopathic infertility. Up to now, there are very little molecular analyses that confirm this hypo-
thesis. The estrogen dependent cellular trans-
duction pathway may be one of the systems responsible for proper differentiation, devel-

ever and pregnancy. It was shown that proper spermatogen-

esis is strictly controlled by estrogen depend-

ing systems including ER alpha and beta, GPR30 and aromatase. The study population consisted of 80 men classified to group of patients with idiopathic infertility. Semen 
analysis was performed according WHO classification. Study of estrogen pathway markers (aromatase, estrogen receptors alpha and beta, GPR30) at mRNA level was carried out using Q-RT-PCR method. Localization of ER alpha and beta was performed using confocal microscopy. Statistically significant positive correlation was found between mol-

lity of sperm and transcript levels of ERs and GPR30. Confocal microscopy analysis revealed ERs localisation in midpiece and tail of spermatozoa suggesting their role in the regulation of mitochondrial function. Al-
terred expression of studied receptors can dis-
turb the hiperactivation process of spermato-
zoa in the woman reproductive tract. There was no association between the transcript levels of studied receptors and embryo qual-
ity and pregnancy rate. Aromatase mRNA expression was negatively correlated with percentage of abnormal spermatozoa and embryos arrested in development. Why the expression of molecular markers in estrogenic pathway is altered in group of men with idiopathic infertility still remains an open question. Our observation indicates that molecular markers such as ERs, GPR30 and aromatase may be utilizable factors in patho-
genesis study of idiopathic infertility.

This work is supported by the Polish Minis-
try of Science and Higher Education (grant No. PBZ-MEIN-8/2/2006).

**Objectives** To assess the relation of Sertoli cell morphology to histopathological and hormonal changes in azoospermic cases.

**Subjects and Methods** 106 males di-

vided into: non-obstructive azoospermia (NOA) (n = 84) and obstructive azoospermia (OA) (n = 22) as controls. They were sub-
ject to semen analysis, serum hormonal assay including follicle stimulating hormone (FSH), luteinizing hormone (LH), testoster-
one (T), prolactin (PRL) and testicular bi-

opsy for histopathology.

**Results** NOA cases with hyposperma-
genesis or germ cell arrest had no serum hor-

monal abnormalities. Sertoli cell only (SCO) pattern was associated with elevated serum
FC 21-6 Male infertility
Follicle-stimulating hormone receptor polymorphism and seminal anti-Müllerian hormone in fertile and infertile men

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Follicle-stimulating hormone (FSH) is fundamental for Sertoli cell function stimulating spermatogenesis and follicular growth by a specific receptor (FSHR). This work aimed to investigate the occurrence of Asn and Ser FSHR gene variants and its relationship with seminal anti-Müllerian hormone (AMH) among normozoospermic and infertile oligo-asthenozoospermic (OAT) males. 82 Caucasian males grouped into normozoospermic healthy controls (n = 30) and infertile OAT males (n = 52). FSHR gene variants were determined by DNA from anti-coagulated blood and underwent polymerase chain reaction (PCR) amplification and electrophoresis in detecting amplification products. AMH in seminal plasma was determined by ELISA. The results showed that the frequency of FSHR gene variants among fertile men was 46.7% Asn/Asn (N680S), 33.3% Asn/Ser, and 20% Ser/Ser, whereas among OAT men were 34.6%, 38.5% and 26.9% respectively with nonsignificant differences. Seminal AMH was significantly higher in fertile than infertile OAT men. There was significant increase in seminal AMH with Asn/Asn variant of FSHR gene than those with Asn/Ser or Ser/Ser. It is concluded that FSH gene variants showed no difference in distribution between fertile or infertile OAT men. However, when correlated with seminal AMH values, there was an increase in Asn/Asn in men with high seminal AMH.

FC 21-7 Male infertility
Do copy number variants play a role in spermatogenic failure and male infertility?

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Introduction Male infertility accounts for about 50% of couple infertility with the most prevalent phenotype of reduced sperm production. The causes for the azoo- or severe oligozoospermia remain frequently obscure with around 30% of idiopathic infertility and 40% with not sufficient/uncertain causes (e.g. varicocele). We hypothesise that the number or specific patterns of CNVs may disturb chromosome synopsis during meiosis and cause meiotic incompetence responsible for reduced sperm output. On the other hand, specific CNVs might de-mask mutations in individual genes important for spermatogenesis.

Subjects and Methods Men with idiopathic infertility (where known causes had been ruled out) were selected retrospectively. So far, DNA extracted from peripheral blood of 64 controls with normal spermatogenesis and 43 patients with severely reduced sperm concentration/count have been analyzed by array CGH using the 244A Array Set (Agilent Technologies).

Results The mean number of CNVs of 9.9 ± 2.7 in patients and 10.6 ± 3.0 in controls was comparable. Neither was the mean quantity of DNA gains and losses different between patients and controls. The distribution of CNVs according to their size was equivalent in both groups. When only patient specific CNVs (not found in controls) were analyzed, an overrepresentation of X-chromosomal variants, mostly duplications, became obvious. Presently, no recurring patient-specific CNVs have been found.

Conclusions To date, no indication for a substantial involvement of CNVs in the pathogenesis of male infertility could be established, but the overrepresentation of X-chromosomal CNVs requires further investigation. Currently, the numbers of patients and controls are being increased to allow more in-depth analyses (e.g. cluster analysis of semen and hormone parameters).

Funding The study was supported by the Deutsche Forschungsgemeinschaft Research Unit „Germ Cell Potential” (FOR 1041/TU 298/1-1).

FC 21-8 Male infertility
The beneficial effect of Tamoxifen on sperm recovery in infertile men with non obstructive azoospermia

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Background About 10% of infertile men have azoospermia. Until 2 decades ago there was no chance of fertility for them. After introducing microinjection (ICSI) many of these men get the chance to be father. But still in many cases of non-obstructive azoospermia, we are not able to find sperm for ICSI. Medications may be able to increase the chance of finding sperm in tests samples. So in this study we evaluated the effect of Tamoxifen citrate on the results of sperm recovery from testis tissue in infertile men with non-obstructive azoospermia.

Methods and Materials 32 azoospermic infertile men with proved non-obstructive azoospermia were selected. Tamoxifen given to them for 3 months. Semen samples and in cases of azoospermia second tests biopsy was taken and the results were compared with the first samples. 12 patients with azoo- spermia who had tests biopsy before evaluation were also selected as control group and TESE was done for them after 3 months.

Results According to first tests samples, 13 patients had hypospermatogenesis, 9 had maturation arrest and 10 patients sertoli cell syndrome. After Tamoxifen treatment 6 patients showed sperm in their ejaculates. Of other patients all in hypospermatogenesis group, 75% in maturation group and 20% in sertoli cell group showed sperm in their second tests samples. In control group we only found sperm in two patients with hypospermatogenesis (p = 0.002).

Conclusions Our study showed that of patients with non-obstructive azoospermia with anti-estrogenic drugs like Tamoxifen can improve the results of sperm recovery in tests samples, and so increase the chance of pregnancy by microinjection.
Monday, September 13

M-001 ART biology
Can the foetus be distinguished from the newborn?
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There has been a substantial increase in the demand for assisted reproduction technology (ART) in aged women. Besides IVF can largely overcome infertility in younger women, but it does not reverse the age-dependent decline in fertility. Women with the metabolic syndrome are known to be at especially high risk for cardiovascular disease (CVD). The prevalence of the metabolic syndrome increases with menopause and may partially explain the apparent acceleration in CVD after menopause. The transition from pre- to post-menopause is associated with the emergence of many features of the metabolic syndrome, including a shift toward a more atherogenic lipid profile and increased glucose and insulin levels, is associated with an increased risk for future symptomatic peripheral artery disease, is associated with incident myocardial infarction and stroke and is linked with sub-clinical inflammation. Metabolic syndrome is in women linked to increased risk for peripheral artery disease (PAD). Single or multiple arterial stenoses produce impaired hemodynamics at the tissue level in patients with peripheral arterial occlusive disease (PAOD). Arterial stenoses lead to alterations in the distal pressures available to affected muscle groups and uterus and to blood flow. If this condition is not good for: Women in many years to reproduction. Who is it good for?

The emergence of these risk factors may be a direct result of ovarian failure or, alternatively, an indirect result of the metabolic consequences of central fat redistribution with oestrogen deficiency. Anyway the result can be an abortion. The fetus is different from the newborn? Where is the limit? Can the foetus to have more risks than the woman?

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M-002 ART biology
The first attempts of cloning somatic cells (therapeutic cloning)
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Probably, embryonic stem cells production from the inner cell mass of blastocyst, originating from nuclear material of somatic cell of a patient, who needs cell therapy for his disease (for example, diabetes), and it’s further differentiation will be very important in the future.

We made an attempt to do the first steps to solve this problem.

In our study we used 47 enucleated oocytes, received from the patients in IVF program after getting their permission. As a nucleus donor we used granulacea cells (7) and epitheliunm of larynx (40).

The activation of oocyte cytoplasm with a somatic cell was successful in 24 cases (51%). Among the embryos originating from the nuclei of granulacea cells 3 reached 2-cell stage and 1 reached 4-cell stage. Among the embryos originating from the nuclei of epitheliunm of larynx 1 reached 12-cell stage, 2–8-cell stage, 2–4-cell stage.

In other words, even without sufficient experience we received cloning embryos. Further research can significantly improve our results.

M-003 ART biology
Gonadotrophins-mediated dynamic changes in cytoplasmic and nuclear maturation in vitro in bovine oocyte
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Introduction In order to eliminate oocytes are exposed to a high level of gonadotropins (Gn) in follicular fluids during clinical super-ovulation. The aim of this study was to evaluate the effect of Gn in a dose-response manner based on an animal model.

Material and Methods Experiments were designed according to current popular stimulation protocols, i.e. Bravelle only (B), combination of Bravelle and Menopur (B+M) or Reprofem (B+R). Bovine oocytes were matured in medium supplemented with 75, 750, 7500 or 75 000 mIU/ml Gn or combination of Gn in vitro for 24 h. In normal control group (NL), no Gn was contained. Then spindle and chromosomal configurations, dynamic changes of cortical granules (CGs) and mitochondria were evaluated by immuno- staining and confocal microscope, while the nuclear status was observed by PI staining.

Results Only 38.46% of the oocytes in NL matured to MII stage. In B groups, the maturation rates were 59.48%, 52.60%, 58.33% and 49.64%, respectively. In B+M groups, they were 61.11%, 58.52%, 50.00% and 44.29%, respectively. And in B+R groups, they were 55.26%, 53.97%, 51.47% and 40.83%, respectively. Low to moderate dose Gn or Gn combination improved nuclear maturation while extremely high dose of Gn did not significantly improve maturation rate. During IVM of bovine oocytes, mitochondria formed larger clusters and migrated more centrally and CGs dispersed. 27.27% and 19.05% of the oocytes in NL had clustered mitochondria distribution pattern and dispersed CGs, respectively. Supplement of any dose of Gn or Gn combination improved the migrations of mitochondria and CGs. Besides, most oocytes from the 75000 mIU/ml groups had clustered mitochondria and dispersed CGs. 55.26% of the oocytes in NL had normal spindle and chromosomal configurations. There were no significant differences between 75, 750, 7500 mIU/ml groups and NL. However, in high dose of 75000 mIU/ml groups, only 19.05% (B) and 11.76% (B+M) of the MI oocytes had normal spindle. In B+R group, no normal spindle was detected at all.

Conclusions Results demonstrated that Gn could improve dynamic changes of mitochondria and CGs. Low to moderate dose of Gn could also improve nuclear maturation. However, extremely high dose of Gn had no accelerating effects on nuclear maturation. Instead, it induced spindle and chromosomal abnormalities. Our study highlights the importance of judicious use of Gn and can be applied to clinical stimulation protocols to reduce the potential risks.
M-004 ART biology
Age-related morphologic and microsomal variations in Syrian hamster ovaries and oocytes and enhanced fertility rate by autologous mitochondrial microinjection

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Aging ova have decreased function as judged by fertilization, implantation and live healthy births. Mitochondria are linked to this mal-function due to reduced replication and increased mtDNA mutations in aging oocytes. Smooth endoplasmic reticulum (SER) may also be part of oocyte cytoplasmic senility. We have evaluated superovulated ovarian follicle number, egg production and microsomal variations in mitochondria and SER between young (2–4 mos.) and old (12–18 mos.) Syrian hamsters. Fertilization and embryo development was assessed following autologous mitochondria microinjection followed by in vitro fertilization. Experimental animals were superovulated, ovaries collected for primordial/primary follicle counts and oocyte masses collected for microscopic analysis by established techniques. Mitochondria were purified from platelets in an autologous blood sample followed by autologous mitochondrial ICMI into female ova and IVF. H&E staining of ovarian tissue from two animals evaluated through serial sections established a 58% decrease in primordial/primary (P1) oocytes in old versus young hamster ovaries (p ≤ 0.05). P1 follicles were GV+ and granulosa cell appropriate. Oocytes were reduced by 40% from superovulated old (avg. 32, n = 13) hamsters (p ≤ 0.05). Ultrastructural components of oocytes showed young hamsters having more mitochondria and smooth endoplasmic reticulum (SER) than old hamsters. All oocyte mitochondria had large amounts of electron dense areas, but old ova had reduced number of distinct cristae and an increased electron dense mitochondrial area. Young ova had no collapsed SER while old ova were replete with areas of collapsed, non-luminal SER. Non-luminal SER were adjoined and contiguous with normal-looking SER in the old oocytes. Ova from 13 young and 20 old females (32x) gave 91% young ova predominantly expanded while 80% old ova displaying same. Fecundity declined 50% in our old compared to young hamsters (n = 8). Autologous mitochondria-microinjected (m-m) oocytes produced 44% (n = 19) blastocysts compared to 33% old control oocytes (n = 12) and 43% sham old oocytes. Autologous m-m oocytes had 0 blastocysts. Young control/sham oocytes (n = 6) produced 50% blastocysts. Results support oocyte depletion as aging occurs and morphological changes of mitochondria and SER in oocytes contributing to age-related fertility decline.

M-005 ART biology
ICSI-mediated gene transfer using human sperm

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Objective In this study, the possibility of applying gene transfer methodologies to the human germline is explored. The objective is to obtain porcine embryos that incorporate and express an exogenous gene (EGFP) by the use of human sperm as vectors for the gene transfer.

Materials and Methods Immature oocytes were collected from porcine ovaries. Maturation was carried out using the standard procedures. Mature oocytes were evaluated through visualization of the first polar body and were used for ICSI. The plasmid used was pCX-EGFP, which contains an enhanced green fluorescent protein gene (EGFP) under the shimeric cytomegalovirus-IE-chicken β-actin enhancer-promoter control. This plasmid permits a rapid and efficient detection of exogenous expression. Samples of human and porcine semen were obtained. Sperm-exogenous DNA (pCX-EGFP) co-incubation was carried out and sperm cells were used for ICSI. A group of injected oocytes (n = 29) was activated artificially and placed in HTF to permit extrusion of their second polar body. After activation, presumptive zygotes were transferred to a 100 µl droplet containing 6-dimethylaminopurine (DMAP) (D2629 Sigma Chemicals Company St. Louis, MO, USA) in HTF for 3 hours. A parthenogenetic control was performed.

In another control only exogenous DNA was injected followed by parthenogenetic activation (n = 35). Injected and parthenogenetically activated oocytes were cultured. Second polar body extrusion and cleavage were 24 and 48 hours post ICSI respectively. To determine EGFP expression, the embryos were analyzed by fluorescence microscopy, exposing them briefly to blue light. To determine the number of embryo blastomeres obtained and discard cellular fragmentation, the embryos were stained. The observation was carried out using ultraviolet light with an epifluorescence microscope.

Results A total of 212 porcine oocytes were maturated in vitro; 55 of them were injected by ICSI, with human spermatozoa incubated with pCX-EGFP. The expression of the EGFP protein in the chemical activated group was 24% (22/92) and the main group was 18% (5/29) and 5% (1/26) respectively. This expression was determined by fluorescent microscopy.

Conclusions To our knowledge, this is one of the few publications presented to produce exogenous DNA expression in embryos by ICSI-mediated gene transfer using human sperm as vectors. In the future this technique combined with gene targeting could be effective to transport therapeutic genes allowing the treatment of monogenic diseases during fertilization.

M-006 ART biology
Tubal Hydration

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Tubal Hydration is an noninvasive method to restore the tubal potency or tubal spasm. Nearly 25% unfortunate couples are childless due to tubal block. Currently the following two methods like IVF and ICSI are being practice mostly for the treatment of infertile couples. These methods are completely invasive, costly and temporary. Economically mid level countries like Bangladesh cannot afford this luxury procedure. More over socio religious factors are working against these. The most common causes of tubal blocked are M.R (repeated), D/C, unsafe delivery, ligation etc. Moreover the percentage of tubal block are rising faster. Concerning this, BIMS is working with tubal hydration since 2001 to restore for tubal potency and to bring invaluable smile at the lives of unfortunate mother. We found that pregnant rate with take home baby is quiet encouraging. To my opinion tubal hydration practice should be one of the options for treatment of tubal block. More and more study is needed.

M-007 ART biology
A combination of ionomicine and 6-dimethylaminopurine effectively produces human parthenogenones with one haploid pronucleus and rescues human unfertilized oocytes after ICSI

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Objective Of all ICSI cycles, failed fertilization occurs in 3% and the mainly cause is due to the inability of the sperm to activate oocytes. The objective of this study is to investigate the parthenogenetic activation with a combination of ionomicine and 6-dime-thylaminopurine as a mean of producing human parthenogenones with one haploid pronucleus and to determine whether this activation could rescue human unfertilized oocytes after ICSI.

Materials and Methods To determine the effectiveness of parthenogenetic haploid activation, mature human oocytes (n = 10) were activated using 5 µM ionomicine for 5 minutes and placed in (HTF) for 3 hours to allow their second polar body extrusion. They were subsequently transferred to a drop of HTF with 1.9 mM 6-dimethylaminopurine for 3 hours. Conventional ICSI was performed in 18 oocytes, the ones that failed to fertilize spontaneously 18 hours after ICSI (n = 5) were assisted with haploid activation to induce fertilization. In all treatments, after washing the oocytes, they were incubated for up to 72 hours in HTF medium. Evidence of activation (pronuclear formation) and cleav-
Results

The pathogenetic activated oocytes showed a haploid activation rate (1PN, 2CP) of 100% (10/10) and a cleavage rate of 90% (9/10), with an average development of 8 cells in 72 hours. The conventional ICSI group showed a fertilization rate (2PN, 2CP) of 66.7% (12/18) and a cleavage rate of 100% (12/12). Finally the oocytes that failed to fertilize after ICSI and subsequently were activated showed a fertilization rate (2PN, 2CP) of 80% (4/5) and a cleavage rate of 75% (3/4), with an average development of 8 cells in 72 hours. Preliminary chromosomal analysis of the embryos with 2PN 2PB showed a normal diploid set of chromosomes (n = 46).

Conclusions

In conclusion, ionomicine followed by 3 hours of incubation previous to fertilization and subsequent 3 hours of egg activation can be used as a safe supplement in sperm wash medium to improve the sperm function under in vitro conditions.

M-009 ART clinical Comparison of outcomes of gamete intra-Fallopian transfer and zygote intra-Fallopian transfer techniques accompanied with in-vitro fertilization

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Background

Infertility is when a couple fails to conceive despite having regular unprotected sex over one year. Infertility is affecting an estimated 10–15% of all couples.

At present, many assisted reproductive techniques (ART) such as gamete intra-Fallopian transfer (GIFT), zygote intra-Fallopian transfer (ZIFT) and in-vitro fertilization (IVF) are developed. To evaluate outcomes and efficacy of GIFT and ZIFT techniques accompanied with IVF, this survey was conducted.

Methods

In a retrospective analytical study, the medical records of 202 infertile couples referred to Infertility Department of Sarem Women’s Hospital that were treated with assisted reproductive techniques (GIFT and ZIFT accompanied with IVF) were evaluated and outcomes of the techniques were compared.

Results

GIFT technique was performed on 110 cases with result of 30 (27.3%) chemical pregnancy and 19 (17.3%) clinical pregnancy. 74 cases was treated with GIFT accompanied with IVF techniques that 28 (37.8%) cases had chemical pregnancy and 14 cases (18.9%) had clinical pregnancy. ZIFT technique was performed on 15 cases with result of 0 (0.0%) chemical pregnancy and 0 (0.0%) clinical pregnancy. Only 3 cases was treated with ZIFT accompanied with IVF techniques that neither chemical pregnancy nor clinical pregnancy was seen (0.0%). There was no statistically significant difference of pregnancy rate by assisted reproductive techniques. In GIFT treated group, 7 (6.4%) cases had abortion and only 10 (9.1%) pregnant cases had delivery. In GIFT accompanied with IVF treated group, 6 (8.1%) cases had abortion and 8 (10.8%) pregnant cases had delivery. No delivery was seen in ZIFT and ZIFT accompanied with IVF treated groups. There was no statistically significant difference of delivery rate by assisted reproductive techniques.

Conclusion

Although, no statistically significant difference of pregnancy and delivery rate by assisted reproductive techniques was seen, this study shows that, performing GIFT accompanied with IVF techniques may improve the outcomes of pregnancy (chemical and clinical) and delivery rate in assisted reproductive techniques.
semination was performed 34–38 hours after hCG administration. Follicles monitoring was performed by ultrasonography just before insemination. Patients with at least one collapsed follicle categorized as oocyte ovulated group (group 1) and those with any collapsed follicles were placed on unovulated group (group 2).

**Results** There are no statistically significant differences among two groups with respect to female age, cause of infertility, mean number of follicles of >15 mm in diameter on the day of hCG administration, or mean number of total motile spermatozoa inseminated. The total pregnancy rate per patient/patient cycle was 15%. Group 1 had 21 (13.5%) in 156 patients and group 2 had 25 (16.6%) in 151 patients. The difference between two groups was not significant.

**Conclusion** It does not need to monitor the ovulation status at the time of insemination in IUI procedure.

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**M-012 ART clinical**

**The role of varicocelectomy on intracytoplasmic sperm injection**


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**Introduction** Assisted reproductive technologies (ART) are important for the management of couples with male factor infertility associated with varicocele, even in cases of men who underwent varicocelectomy. The aim of the study was to evaluate the contribution of varicocelectomy, proposed before infertility treatment, in improving the sperm quality and consequently the outcomes of ART.

**Methods** Data from 248 patients submitted to intracytoplasmic sperm injection (ICSI) cycle, as a result of male infertility associated with varicocele, were retrospectively evaluated and subdivided in 2 groups: men who did not perform surgical repair (Group 1, n = 79) and those who did perform varicocelectomy (Group 2, n = 169) before ICSI. Ejaculated samples were assessed regarding semen parameters according to WHO, and Tygerberg’s strict criteria was used to carry out sperm morphology analysis. The groups were compared regarding ICSI outcome.

**Results** No significant differences were found between the 2 groups regarding demography and cycle’s general characteristics. However, our results indicate that paternal age (36.1 ± 5.5 vs 37.8 ± 4.7; p = 0.031) and period of infertility (2.7 ± 0.3 vs 6.0 ± 4.9; p < 0.001) were significantly higher in men who underwent varicocelectomy. Although semen volume was higher in Group 1 (4.9 vs 4.3 ± 0.3 vs 2.5 ± 0.14; p = 0.004), sperm concentration (30.08 ± 4.01 vs 24.1 ± 2.42; p = 0.138), progressive motility (38.2 ± 2.69 vs 38.7 ± 2.08; p = 0.881), as well as normal morphology (2.6 ± 0.44 vs 2.4 ± 0.37; p = 0.7202) were similar between groups. Fertilization rate was significantly higher in Group 1 (73.2% vs 64.9%; p = 0.0377). However, no differences were observed on pregnancy (31.1% vs 30.9%; p = 0.9806), implantation (22.1% vs 17.3%; p = 0.3882) and miscarriage rates (21.7% vs 23.9%; p = 0.8401) in Groups 1 and 2.

**Conclusion** Although varicocelectomy has become the most commonly performed operation regarding male infertility, it benefits on the restoration of testicular function, as well as fertility potential are controversy. Our results suggest that this surgical procedure does not have any significant impact on ICSI outcomes. In addition, our results showed that when submitted to an ART, men who underwent varicocelectomy are older, with higher period of infertility than those not submitted to varicocele repair. ART could be considered an important alternative in the management of couples with clinical varicocele, without benefit of previous surgical treatment.

**Funding** none.

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**M-013 ART clinical**

**Correlation of serum anti-Müllerian Hormone level and ovarian response in IVF**

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**Background** Serum anti-Müllerian Hormone (AMH) has been introduced as a novel measure of ovarian reserve and might be used as ovarian response indicator in IVF. The objective of this study is to determine serum AMH level and its correlation with age, basal FSH level, ovarian response, and total dose of gonadotrophin used in IVF.

**Material and Methods** This study was a cohort study. We followed up 80 infertile couples who underwent their first 80 IVF cycles in Cipto Mangunkusumo hospital, Jakarta from August 2008 until February 2009. Basal serum AMH and basal serum FSH were measured on the day-3-menstrual cycle in the beginning of IVF cycle. Ovarian response was indicated by the number of mature oocytes which were retrieved in IVF. Mature oocytes were metaphase II oocytes which had extruded first polar body. The total dose of recombinant FSH (rFSH) needed for COH protocol for IVF was also evaluated.

**Results** Serum AMH level was inversely correlated with age (p = 0.002; r = -0.414) and basal FSH level (p < 0.0001; r = -0.468). There was significantly positive correlation between serum AMH level and the number of mature oocytes retrieved (p = 0.005; r = 0.368). There was inverse correlation between serum AMH level and the total dose of rFSH used in COH protocol for IVF (p = 0.026; r = -0.324). The AUC serum AMH level for good responders in IVF was 0.729 (p = 0.004) with cut-off value ≥ 3.33 ng/ml (63% sensitivity and 65% specificity).

**Conclusion** Serum AMH level was correlated significantly with age, basal FSH level, ovarian response, and total dose gonadotrophin used in IVF.

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**M-014 ART clinical**

**The effect of transdermal estradiol patch or gel for endometrial preparation on the outcome of frozen/thawed embryo transfer in Japanese women**


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**Objectives** To avoid the risk of multiple pregnancy, selective single embryo transfer by frozen/thawed embryo transfer (FET) has become more popular in Japan. For endometrial preparation for FET, transdermal estradiol product is recommended as oral one has a risk of venous thrombosis. Recently in Japan, transdermal estradiol gel became available to use. The aim of this study is to compare the effects of transdermal patch and gel on the outcome of FET in Japanese women.

**Materials and Methods** Endometrium was stimulated by administration of 17β estradiol patch (n = 133) or gel (n = 65) from day 4–5 of menstrual cycle and the stimulation was continued until endometrial thickness evaluated by transvaginal ultrasonography reached 8 mm or more. Then, progesterone was administered to support the luteal function. FET was performed at day 3–5 after progesterone administration. Serum estradiol, progesterone and hCG were measured at before and after FET and at 5–8 weeks gestation. Pregnancy and abortion rates were also evaluated.

**Results** Pregnancy rate, implantation rate and abortion rate in patch and gel groups were 24.2% (31/128) and 26.3% (15/57), 18.9% (31/164) and 19.5% (15/77), 3.2% (1/31) and 13.3% (2/15), respectively and were not statistically significant between both groups. Serum estradiol level at the day of FET was significantly higher in gel group (325 ± 299 pg/ml) than that of patch group (218 ± 146 pg/ml) (p < 0.001). However, no significant differences were seen in serum estradiol, progesterone or hCG levels at any time thereafter. Endometrial thickness at the day of FET was 10.2 ± 2.0 mm in patch group and 9.7 ± 2.5 mm in gel group. Duration of endometrial development until endometrial thickness reached 8 mm was not also different between both groups.

**Conclusions** Although serum estradiol level was higher in gel groups, there was no significant difference in clinical outcomes.
M-015 ART clinical
Management of poor responders: Comparison of modified natural, soft stimulation and microdose flare-up protocols
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Background The management of poor responders has been the major obstacles in spite of rapid development of assisted reproduction technology (ART). Although the various protocols have been used for this group, no single protocol demonstrated the superiority to others. The aim of this retrospective study was to compare the efficacy of modified natural, soft stimulation and microdose flare-up protocols.

Materials and Methods Study subjects was composed of poor responders (number of oocytes retrieved being less than or equal to 5) who have had ART undergoing controlled ovarian hyperstimulation and IVF / ICSI with modified natural (n = 17), soft stimulation (n = 16), and microdose flare-up protocols (n = 22). The main clinical outcomes included the number of oocytes retrieved, the number of transferred embryos, on-going pregnancy rate and the cancellation rate.

Results Demographic data including the mean age of the patient and the partner, causes and duration of infertility, BMI, basal hormone levels, antral follicle count have not shown difference statistically among three groups. No difference in the number of oocytes retrieved, the number of transferred embryos, ongoing pregnancy rate, and the cancellation rate was observed in study population. However, the mean dosage of gonadotropin used and days of stimulation was significantly lowest in women treated with modified natural protocol and the second lowest in those treated with soft stimulation protocol (p < 0.001 and p < 0.001, respectively).

Conclusions Considering management of poor responders in ART, modified natural protocol and soft stimulation protocol might be adequate options with the advantages of shorter days of stimulation and lower costs for gonadotropin.
M-018 ART clinical
Hormonal microsurroundings and oocytes fertilization potential in women with hyperandrogenia in dependence on the outcome of IVF cycles
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Introduction The aim of the study was to compare the hormonal profile of oocytes microsurroundings and their fertilization parameters in patients with basal high testosterone (T) level who achieved or not pregnancy at the IVF/ICSI cycles.

Materials and Methods 52 women aged of 31.1 ± 2.5 years were included in the study. Patients were divided in 2 groups, depending on their androgenic status: normo-androgenia (NA, n = 38) and hyperandrogenia (HA, n = 14). All patients underwent a controlled ovarian stimulation with FSH in GnRH analogue protocols. The levels of T, estradiol (E2), sex hormone binding globulin (SHBG), leptin in follicular fluid (FF) from the largest follicles, the level of leptin before ovarian stimulation, content of E2 in blood serum on Day of hCG administration (Day 1) and on Day of embryos transfer (Day 5) were determined with ELISA kits. The index of free androgens (IFA) was calculated. The number of obtained oocytes and embryos, the number and rate of morphologically good embryos, the pregnancy rate were counted. The percentage of patients with a low incidence of OHSS. Recently, a novel combination gonadotrophin product, with a 2:1 formulation of follitropin alfa and luteinising hormone (150 IU recombinant human follicle-stimulating hormone [FSH]: 75 IU recombinant human luteinizing hormone [LH]; Pergoveris) has become available for induction of follicular development in women with severe LH and FSH deficiency. The pregnancy rate in this group was 35 vs 50% in group NA. While comparing women with HA who achieved pregnancy (n = 5) or not (n = 9), we revealed that serum E2 levels on Day 1 and Day 5 were 2 times higher at patients who became pregnant (p < 0.05) and were equal to its levels at women with NA. The SHBG and leptin levels, the number of non-athretic oocytes and good embryos were also significantly greater at pregnant HA women and did not differ from NA pregnant patients, although the level of T and IFA were identically higher in both pregnant and non-pregnant HA patients.

Conclusion The probability of achieving pregnancy at women with HA, which have after controlled ovarian stimulation, similar to NA patients, higher levels of E2, SHBG, LH and E2 in serum is increased.

M-019 ART clinical
Controlled ovarian stimulation using a novel 2:1 FSH: LH gonadotrophin preparation – results from routine clinical practice
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Objective Controlled ovarian stimulation for assisted reproduction is widely used as a prerequisite for ensuring the development of a cohort of mature follicles and hence oocytes for fertilization. Gonadotrophin preparations, primarily FSH containing, have been demonstrated to be effective and associated with a low incidence of OHSS. Recently, a novel combination gonadotrophin product, with a 2:1 formulation of follitropin alfa and luteinising hormone (150 IU recombinant human follicle-stimulating hormone [FSH]: 75 IU recombinant human luteinizing hormone [LH]; Pergoveris) has become available for induction of follicular development in women with severe LH and FSH deficiency. The SHBG and leptin levels, the number of oocytes fertilization potential in women with HA, which have after controlled ovarian stimulation, similar to NA patients, higher levels of E2, SHBG, LH and E2 in serum is increased.

Materials and Methods Patients undergoing COS for ART with 2:1 FSH: LH included in the protocol and having oocyte retrieval in our clinic between October 2008 and December 2009 were analyzed. The mean (SD) age, 30.4 (3.5) years; BMI, 22.5 (4.7); basal FSH 6.0 (4.5) IU/L (n = 1016); and antral follicles < 11 mm, 7.7 (4.2) (n = 1015). The mean (SD) physician-recommended starting r-hFSH dose was 161.7 (44.4) IU (n = 1018); range: 75–450 IU), while the CONSORT calculated dose was 135.1 (40.0) IU (n = 979; range 112.5–300 IU). Starting dose was increased in 559 (58.4%), decreased in 136 (14.2%) and remained unchanged in 263 (27.4%) patients. Of the 263 patients who received the CONSORT dose, 202 (76.8%) were undergoing their first ART cycle, 29 (11.0%) their second, 16 (6.1%) their third, and 16 (6.1%) had undergone > 3 previous ART cycles. A contraceptive pill was given in 42.8%, a GnRH antagonist in 20.9% and a GnRH agonist in 79.1% of cycles (97.6% of which used a long GnRH agonist protocol). Mean (SD) duration of stimulation was 11.0 (2.1) days and r-hFSH dose was 1597.5 (765.4) IU. hCG was given in 260 (98.9%) cycles, each embryo transfer was 2.0 (0.40), Implantation rate per embryo transfer was 27.1%, with a clinical pregnancy rate per transfer of 44.4%, a clinical pregnancy rate per started cycle of 39.2%, and a miscarriage rate of 10.7%. No case of severe OHSS grade III was observed, but 2 cases of OHSS grade II led to hospitalization (0.76%). Three extrauterine pregnancies (1.29%) were reported.

Conclusions Routine use of the CONSORT calculator for individualized starting doses of r-hFSH for COS during routine ART procedures was effective in achieving clinical pregnancies and was associated with a favourable safety profile.
M-021 ART clinical
Triggering oocyte maturation with GnRH agonist or hCG in GnRH antagonist cycle undergoing IVF treatment: A new strategy for preventing OHSS in women with polycystic ovary syndrome/polycystic ovaries

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Triggering of final oocyte maturation with gonadotrophin-releasing hormone agonist (GnRHα) has been shown to prevent ovarian hyperstimulation syndrome (OHSS), but a detrimental effect on clinical outcome was also observed in GnRHα triggered cycle compared to hCG for a controlled ovolution stimulation (COS) co-treatment with GnRH antagonist. We employed a new strategy that GnRHα or hCG was chosen to trigger final oocyte maturation in PCOS/PCO patients, who was at OHSS high-risk undergoing IVF/ICSI with GnRH antagonist cycles. The aim was to avoid cycle cancellation in OHSS high-risk patients and simultaneously secure the clinical outcome. 37 PCOS/PCO patients were prospectively enrolled to have final oocyte maturation triggered with 0.2 mg dinapreline i.m. (GnRHα group) or 5000-10000 IU hCG (hCG group) according to the number of follicles and estradiol level when 3 leading follicles ≥ 17 mm in diameter. Patient with ≥ 25 follicles ≥ 10 mm or E2 ≥ 3000 pg/ml were assigned to GnRHα group (n = 21), otherwise the patient was assigned to hCG group (n = 16). Luteal phase support were used by 60 mg/d progesterone i.m. combined a single bolus of 2000 IU hCG on the oocyte retrieval day in GnRHα group and 60 mg/d progesterone i.m. in hCG group. There were significant differences in aspirated follicles (32 ± 9 follicles vs 21 ± 5 follicles), retrieved oocytes (18 ± 7 oocytes vs 12 ± 8 oocytes), peak of E2 level (3976 ± 1261 pg/ml versus 1853 ± 851 pg/ml) in the GnRHα group and hCG group, respectively. All patients underwent embryo transfer, resulting in a clinical pregnancy rate (CPR) per cycle of 28.6% (6/21) vs 56.3% (9/16) and ongoing PR of 28.6% vs 50.0% in GnRHα group and hCG group, respectively. There was higher CPR and ongoing PR in hCG group than in GnRHα group but significant differences have not reached because of small sample size. One patient developed moderate OHSS in hCG group and no moderate/severe OHSS occurred in GnRHα group. Our results suggested ovulation triggering with GnRHα result in lower CPR and ongoing PR than ovulation triggering with hCG. Luteal support with a single hCG can not completely overcome the detrimental effect. For preventing OHSS and optimizing clinical outcome, we recommend a new strategy that choosing GnRHα or hCG for triggering final maturation base on the number of follicles and peak E2 level in GnRH antagonist cycles for PCOS/PCO patients.

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M-022 ART clinical
Endometriosis pain and infertility

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Setting The pain associated with endometriosis is the most difficult symptom to cope with for most women. It can be constant or cyclical.

Purpose Looking for cause and treatment of endometriosis pain and infertility.

Methods We have performed a review in a worldwide basis on state of the art problem.

Results Firstly how endometriosis causes pain is the topic of much research. Because many women with endometriosis feel pain during or related to their periods, some researchers are focusing on the menstrual cycle in their search for answers about pain.

Which is associated with the disease is not related to the extent or size of the implants. Some women with endometriosis have no symptoms, others have debilitating pain. Women with endometriosis often have several types of chronic pain conditions because their abnormal growths develop a nerve supply that communicates with the brain. Cysts become supplied by sympathetic and sensory nerves that could contribute to both the different types of pain associated with endometriosis and the body’s ability to maintain the disease and so infertility. This nerve supply might also help the abnormally-located tissue survive and grow by setting up its own blood supply. The most common pain sensation, nociceptive pain, results from physical tissue damage, which is experienced through the nervous system. A second type of pain, neuropathic pain, stems from a malfunction in the central and/or peripheral nervous system. Neuropathic pain is different. It is a chronic condition which is caused by damage or dysfunction in the nervous system. There are different treatments as nonsteroidal anti-inflammatory drugs, gnrh agonists, opioid analgesics, hormones, etcetera. Infertility is also in relation to

Conclusions So, nociceptive pain need hormonal or standard treatment and neuropathic pain need treatment too like gabapentin, duloxetine or prescalar neuroectomy and best the combination of both. And meanwhile infertility is waiting for coping with as for delay in pregnancy, which will increase the condition issue.

M-023 ART clinical
Recurrent early abortion: An overview on the management of some unusual factors

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Unexplained infertility, recurrent implantation failure (RIF) in IVF and repeated early abortions (REA) are three unresolved reproductive problems. All the cases included in the study (REA) were subjected to a long list of investigations (Immunologic, infections, chromosomal, hormonal, uterine and metabolic). All the cases had negative test results. Since a good endometrial blood flow is essential for proper implantation, we have adopted in the past (as many other groups did) the empirical use of low dose aspirin, follic acid plus heparin as well as routine luteal phase support. 40 cases of REA were recruited to the above mentioned empirical regimen starting on day 18 of the pre-pregnancy cycle, to continue if pregnancy is documented or to restart again the following cycle. All the cases showed a pretreatment border-line B.P. and or increased uterine artery Resistance index (RI) by power Doppler. The cases were randomized to 2 equal groups of which one received an additional daily dose of Nifedipine retard (calcium channel blocker). Uterine artery blood flow evaluated by RI showed improvement during treatment, which was highly significant among Nifedipine users compared to non-users. After treatment (on day 24 of the cycle) RI was 0.652 ± 0.169 during Nifedipine use vs 0.755 ± 0.132 without the calcium channel blocker (p = 0.04). There was a significant increase in the live birth rate among the Nifedipine group compared to controls (7 vs 4), but the difference was not statistically significant probably due to the small sample size. Two cases aborted in each group. Safety of Nifedipine in early pregnancy is not fully documented but there are no human data on confirmed risks. Only high doses in animal studies reported fetal risks. At present, its use may only be justified if benefits outweigh risks in animals. The patients were counselled about this matter and gave their consent. There were no congenital anomalies detected in the new-born infants. U/S Doppler technology serves an important functional diagnostic tool in infertility management. This is needed to develop pharmacological approaches to improve blood flow impendence and may also prove useful in REA. These may include Nifedipine or nitric oxide donors. The results showed that the selected group had increased impedance to uterine blood flow and that the administration of vasodilators (in addition to empirical therapy) seems to offer a new and beneficial approach to manage such difficult cases.
M-024 ART clinical
Modified natural cycle in vitro ferti-
лизation in poor responder pa-
tients – the last chance before egg
donation; the role of follicle diam-
eter on the day of hCG adminis-
tration in order to improve results
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Objective Modified Natural Cycle (MNC)
IVF may represent an easy, friendly as well
as a cheap approach to treat poor responder
patients – the last chance before egg
transfer. The last chance before egg
donation in poor responder pa-
tients – the last chance before egg
donation. Administra-
tion of hCG at a follicle ≥ 19 mm, may im-
prove the results.

M-025 ART clinical
Early start of gestational diabetes
(GDM): A possible risk factor for
IVF failure
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Aims Infertile patients frequently have
an increased risk for GDM. Predisposing fac-
tors for GDM are e.g. polycystic ovary syn-
drome (PCOS), impaired glucose tolerance
(IGT) or advanced maternal age. Women
with a history of recurrent spontaneous abort-
ations (RSA) are also at risk for GDM because
they have an increased prevalence of insulin
resistance (IR). Possibly recurrent implanta-
tion failure (RIF) is due to early start of GDM
after embryo transfer. The incidence of GDM in
ART patients with an increased risk was
investigated immediately when pregnancy
was achieved.
Methods In this cohort study, 107 women
were investigated immediately when preg-
nancy was achieved. Administration of
treatment in poor responder patients
of hCG administration.
Results Egg collection was performed in
46 out of 82 cycles (50%). In 45 cycles (70%)
at least one oocyte was obtained. Fertiliza-
tion rate was 54% and cleaving embryos for
transfer were obtained in 50% of cycles.
When the follicle diameter was ≥ 17 ≤ 19 mm
on the day of hCG administration, at least
one oocyte was obtained in 78% of those
cycles with fertilization rate of 68%. When
the follicle diameter was ≥ 19.5 mm one oo-
cyte was retrieved in only 60% of the cycles
and fertilization rate was 48%. Pregnancy
was achieved in 7 cases.
Conclusions In poor responder patients
only 1 oocyte is often obtained in IVF cycles
despite large amount of gonadotrophin used
in different protocols of stimulation. For
these patients modified natural IVF cycle is a
feasible alternative with low cost and low
risk despite of high cancellation rate, before
being referred to egg donation. Administra-
tion of hCG at a follicle diameter > 16 mm at least
of the cycle and every 2–3 days until it
reached ≥ 17 mm, may im-
prove the results.

M-026 ART clinical
Effect of oestradiol decrease rate on
clinical outcome during the short
coasting period in high responder
patients treated with GnRH ago-
nist protocol
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Objective To determine the effects of dec-
rement in estradiol (E2) levels on pregnancy
outcome during short coasting among high
responder patients.
Design Retrospective analysis.
Materials and Methods The database was
retrospectively evaluated between 2005 and
2008. High responder patients coasted less
than 4 days were evaluated.
Results A total of 383 short coasted cycles
and 234 controls were analyzed. Groups in
relation to drop in E2, levels were defined as
< 20% (n = 24), 20% to < 40% (n = 30), 40% (n = 38),
the group 1, group 2 and group 3, re-
spectively. There was no difference in pa-
tient parameters between the groups. The
total collected oocytes, total MII and ferti-
zed oocytes and the mean number of embryos
transferred were similar between the
groups. Biochemical pregnancy loss rates were
in group 2 (25%) and group 3 (22.7%) signifi-
cantly higher than group 1 (6.2%) and control
(8.3%) (p = 0.04). There were no significant differences between groups 1,
2, 3 and control group with respect to clinical
pregnancy rates (62.5%, 40%, 44.7% and
56.8%, respectively; p = 0.16), ongoing pregnancy
rates (54.1%, 30%, 34.2% and
47.8%, respectively; p = 0.10) and miscar-
riage rates (13.3%, 25%, 23.5% and 15.7%,
respectively; p = 0.61). Implantation rates
were similar between groups 1, 2, 3 and
control group (48%) (p = 0.85)
Conclusions Controversy exists whether
there is a safe threshold of E2 decrement that
would not jeopardize IVF outcome after
coasting. E2 decrease ≥ 20% during short
casting seems to significantly increase bio-
chemical pregnancy loss rate. Although sta-
istically insignificant, slight differences in
terms of clinical pregnancy, ongoing preg-
nancy and miscarriage rates in patients show-
ing ≥ 20 % drop in E2 levels may help the cli-
M-027 ART clinical
Should cervical erosion interfere treatment be performed initially or not, during infertility management?
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Aim To investigate the influence of cervical erosion treatment on the pregnancy efficacy in infertile women.

Methods A total of 148 infertile women who underwent infertile treatment from January 2008 to December 2008 were investigated. They were divided into 2 groups according to whether they have received operations on cervical erosion. The cervical mucus score and the post-coital test of non-operation women were compared with those post-operative women. The correlation between pregnant result and the style of operation was also identified. The results were evaluated by SAS version 8.0 for Windows.

Results Compared with the infertile women with no operation, women with cervical erosion operation had a significantly higher cervical mucus score (9.10 ± 2.48 vs 9.72 ± 2.47; p < 0.05) in cumulative 3-cycle test. No significant statistical difference was identified in post-coital test in 2 groups. Cumulative pregnancy rate of 3 cycles in operation group was significantly higher than those in non-operation group (16.58% vs 3.26%).

Conclusions Cervical erosion operation before infertile treatment benefits to the infertile women with cervical erosion.

M-028 ART clinical
Letrozole with gonadotrophins improves success rates in PCOS patients undergoing intrauterine insemination compared to other two groups
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Objective To evaluate the use of letrozole with FSH in patients undergoing superovulation and IUI in PCOS group of patients and compare their efficacy with letrozole and gonadotrophins alone.

Design Retrospective study in 3 groups of PCOS patients receiving letrozole only, Letrozole and FSH and gonadotrophins alone and undergoing intrauterine insemination.

Setting Fertility Research Center

Patients PCOS women < 40 years and infertility > 1 year undergoing IUI.

Interventions Letrozole was administered in a dose of 5 mg from Day 3–Day 7 of the cycle in first group of patients. The second group received letrozole 5 mg from Day 3–Day 7 and gonadotrophins from 75 IU IM from Day 5 until the dominant follicle reached 18 mm and ovulation was triggered with 5000 IU hCG. The 3rd group received gonadotrophins alone from Day 3 of cycle till the dominant follicle was 18 mm and ovulation was triggered with hCG 5000 IU. Intrauterine insemination was performed 36 hours and 48 hours after hCG administration.

Main Outcome Measures Follicular response by transvaginal ultrasound was studied. No of follicles more than 18 mm, FSH dose, endometrial thickness and comparing pregnancy rates in 3 groups of superovulation with intrauterine insemination in PCOS groups of patients.

Results Intrauterine insemination was performed in 170 cycles in PCOS patients were conducted in a 3 year period from January 2007 to December 2009. Letrozole alone was used in 59 PCOS women undergoing IUI, letrozole plus gonadotrophins was used in 61 PCOS patients undergoing IUI, and gonadotrophins alone was used in 50 patients with PCOS undergoing IUI. 16 out of the 59 patients receiving letrozole conceived (27.1%), 25 out of the 61 patients undergoing IUI with letrozole and gonadotrophins conceived (40.98%) and 11 out of the 50 patients receiving gonadotrophins conceived (22%). Patients cotreated with letrozole required fewer gonadotrophins, developed more follicles larger than 14 mm. The endometrial response was significant in letrozole plus gonadotrophins group. The pregnancy rate in PCOS undergoing intrauterine insemination with letrozole plus gonadotrophins group was 40.98% compared to letrozole group (22%).

Conclusion The addition of letrozole to gonadotrophins in PCOS patients undergoing IUI decreases gonadotrophin requirement, increases preovulatory follicles, with no negative effect on endometrium but with marked increase in pregnancy rates.

M-029 ART clinical
Large multicenter, observational study of a formulation of follitropin alfa and lutropin alfa with a 2:1 ratio in ART in routine clinical practice
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Objective To evaluate whether cycle day 3 basal quantal blood flow, antral follicle count and anti-Müllerian hormone level in stimulated cycles
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Objective To evaluate whether cycle day 3 basal quantal blood flow, antral follicle count and anti-Müllerian hormone (AMH) level are useful tools to predict diminished
M-032 ART clinical
Efficiency of double preovulatory intrauterine inseminations in cycles of controlled ovarian stimulation

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Aim This is a prospective study, where infertile patients were treated with 2 consecutive intrauterine inseminations (IUI). There are different opinions and answers to the question if repeated IUIs in the same cycle result in higher pregnancy rates due to longer release of spermatozoa within uterine cavity and tubes.

Materials and Methods 173 patients were treated with IUI (97 with unexplained infertility and 76 with male factor). They had total of 346 cycles (194 with unexplained and 152 cycles with male factor). IUI was performed in all of them after applied ovulation stimulation (CC 100 mg per day, 3–7 days of menstrual cycle) and 150–225 IU of pure FSH starting from the cycle Day 6. The patients were randomized into 3 groups: group A included 58 patients with total 116 cycles. These had just one insemination before ovulation, i.e. 36 hours after receiving hCG. Group B comprised of 58 patients with total 116 cycles and they had IUI before ovulation. Group C included 59 patients with 118 cycles and they had IUI performed at 34 and 48 hours after receiving hCG. Data analysis did not reveal significant differences between 3 groups regarding indications, age and average number of motile spermatozoa.

Results Pregnancies were achieved in 37 (21%) women. There were 14 (18.42%) pregnancies in male factor cases and 23 (23.7%) among unexplained infertility cases. In group A, among 58 patients there were 12 (20.7%) pregnancies. In group B, among 58 patients there were 19 (32.7%) pregnancies. In group C, among 59 patients, 6 (10.2%) got pregnant. There is a significant difference (p < 0.001) between group B (repeated IUI at 24 and 34 hours) and groups A and C (with one IUI 34 hours after hCG and with repeated IUI 34 and 48 hours after hCG). Among 37 pregnancies, 28 patients delivered healthy babies (75.6%), 8 had spontaneous abortions (21.6%), and one (2.7%) had extrauterine pregnancy.

Conclusion The study included 173 patients (346 cycles) and compared one insemination before ovulation 34 hours after receiving hCG and double inseminations before ovulation 24 and 34 hours after receiving hCG, as well as double inseminations before and after ovulation, i.e. 34 and 48 hours after receiving hCG; it showed a significant improvement in number of pregnancies. Therefore, we believe that despite increased costs double inseminations before ovulation improves the results and can be applied in routine clinical practice.

M-033 ART clinical
The application of recombinant human albumin in embryo culture

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Introduction Albumin supplements in culture media have multiple physiological functions in embryo development. It is known that heat inactivation procedures may not eliminate prions, which transmit diseases like Creutzfeldt-Jakob disease. At present, most fertility clinics use commercial media in which the serum is replaced with various preparations of human serum albumin (HSA) as a protein source despite the possibility that such supplements may be contaminated with viruses such as HIV, HBV, and HCV, as well as prion proteins. In this study we investigated the application of recombinant human albumin (rHA) in human embryo cultures.

Methods The effects of 0.1% and 0.4% rHA (Vitrolife) in the same culture medium were compared using ICR mouse embryos. Since 0.1% HSA supplements produced significantly higher blastocyst development rates and hatching rates, we used 0.1% rHA for clinical embryo culture and compared the results with our routine laboratory procedure employing 0.5% HSA (GYNEMED). After performing clinical IVF or ICSI, 261 zygotes resulting from IVF and 165 zygotes from ICSI were divided randomly into 2 groups to compare cleavage rates and the yield of good quality embryos on Day 2. Embryos that had 4 blastomeres or more with less than 20% fragmentation were considered to be of good quality.

Results The use of commercial HSA produced a 76.7% (99/129) in vitro fertilization rate, 97.0% (96/99) cleavage rate and 67.7% (67/99) good quality embryo on Day 2 while
the results with rHA were 77.3% (102/132), 95.1% (97/102) and 73.5% (75/102) respectively. When routine HSA was used for ICSI, the fertilization rate was 76.2% (62 cycles, cleavage rate 95.3% (61/64) and 81.3% (52/64) good quality embryos were obtained on day 2, while the results with rHA cultures were 76.5% (62/81), 95.2% (59/62) and 79.0% (49/62) respectively. Thus the use of 0.1% rHA in culture media produced similar results to those obtained with 0.5% HSA supplements, and no statistically significant differences were detected in the parameters analyzed, between the two groups in both IVF and ICSI.

Conclusions At a concentration of 0.1% rHA supplements produced similar fertilization and embryo development on day 2 as commercial 0.5% HSA. However, the advantage of rHA is that it reduces the likelihood of transmitting viral and prion infections.

M-034 ART clinical
Dual embryo transfer: Pro and contra
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Introduction The time of embryo transfer is one of a key point of IVF procedure. The clinical pregnancy rate is higher after embryo transfer on Day 5 at the blastocyst stage. But in some patients prolonged cultivation leads to the termination of embryo development on Day 3 or 4 and transfer cancellation at all. To prevent such an outcome and increase pregnancy rate we started trial with dual embryo transfer: first one on Day 3 and second one Day 5.

Objective Estimation of IVF outcomes before and after dual embryo transfer.

Materials and Methods Retrospective study of 992 consecutive IVF/ICSI cycles in the Affiliated Drum Tower Hospital of Nanjing University Medical School, The Reproductive Medicine Center, Nanjing, China.

Methods Data obtained during nine months from 743 embryo transfers, using a medium containing a high concentration of hyaluronan, were compared with data obtained during the same months one year earlier from 471 embryo transfers using a medium with a low concentration of hyaluronan. The same staff, equipment, culture medium and methods were used during both time periods. All embryo transfers were performed on Day 3. Data were analyzed with a Fisher’s exact test. All significance tests were two-tailed and conducted at the 0.05 significance level.

Results Both groups were similar on baseline variables. The clinical pregnancy rate (CPR), the implantation rate (IR) and the delivery rate (DR) per embryo transfer were all increased significantly with the use of a high concentration of hyaluronan in the transfer medium (CPR, 59.9 % vs 54.1; p = 0.04, OR 1.3, 95 %-CI: 1.0–1.6; IR, 38.8 % vs 34.2%; p = 0.02, OR 1.2, 95 %-CI: 1.0–1.4; DR, 50.1% vs 43.4 %; p = 0.02, OR 1.3, 95 %-CI: 1.0–1.7). The number needed to treat to obtain one extra delivery with high concentrations of hyaluronan in the transfer medium was 15.

Conclusion In conclusion, transfer medium containing increased concentrations of hyaluronan can significantly improve the clinical pregnancy, implantation and delivery rates after day 3 fresh embryo transfer.

M-037 ART clinical
The effectiveness of 6% hydroxyethyl starch solution in reducing the incidence of severe hyperstimulation syndrome in polycystic ovarian disease patients: A prospective cohort clinical trial
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Introduction Hyaluronan is the major glycosaminoglycan present in follicular, oviductal and uterine fluids. The synthesis of hyaluronan increases dramatically by 5 to 6 fold on the day upon which implantation is initiated and it decreases to near basal levels by the next day. Several studies have been performed to assess whether hyaluronan in the human embryo culture system can improve pregnancy and implantation rates. None of these studies used delivery rate as a study endpoint. In order to verify the role of hyaluronan on clinical outcome, especially on the delivery rate, a retrospective controlled study was performed using two groups of patients, one with the transfer medium containing high concentrations of hyaluronan and one with the transfer medium containing low concentrations of hyaluronan.

Methods Data obtained during nine months from 743 embryo transfers, using a medium containing a high concentration of hyaluronan, were compared with data obtained during the same months one year earlier from 471 embryo transfers using a medium with a low concentration of hyaluronan. The same staff, equipment, culture medium and methods were used during both time periods. All embryo transfers were performed on Day 3. Data were analyzed with a Fisher’s exact test. All significance tests were two-tailed and conducted at the 0.05 significance level.

Results Both groups were similar on baseline variables. The clinical pregnancy rate (CPR), the implantation rate (IR) and the delivery rate (DR) per embryo transfer were all increased significantly with the use of a high concentration of hyaluronan in the transfer medium (CPR, 59.9 % vs 54.1; p = 0.04, OR 1.3, 95 %-CI: 1.0–1.6; IR, 38.8 % vs 34.2%; p = 0.02, OR 1.2, 95 %-CI: 1.0–1.4; DR, 50.1% vs 43.4 %; p = 0.02, OR 1.3, 95 %-CI: 1.0–1.7). The number needed to treat to obtain one extra delivery with high concentrations of hyaluronan in the transfer medium was 15.

Conclusion In conclusion, transfer medium containing increased concentrations of hyaluronan can significantly improve the clinical pregnancy, implantation and delivery rates after day 3 fresh embryo transfer.

M-036 ART clinical
A retrospective clinical study on effects of hyaluronan-containing transfer medium on implantation, pregnancy and delivery
The Affiliated Drum Tower Hospital of Nanjing University Medical School, The Reproductive Medicine Center, Nanjing, China

Introduction Hyaluronan is the major glycosaminoglycan present in follicular, oviductal and uterine fluids. The synthesis of hyaluronan increases dramatically by 5 to 6 fold on the day upon which implantation is initiated and it decreases to near basal levels by the next day. Several studies have been performed to assess whether hyaluronan in the human embryo culture system can improve pregnancy and implantation rates. None of these studies used delivery rate as a study endpoint. In order to verify the role of hyaluronan on clinical outcome, especially on the delivery rate, a retrospective controlled study was performed using two groups of patients, one with the transfer medium containing high concentrations of hyaluronan and one with the transfer medium containing low concentrations of hyaluronan.

Methods Data obtained during nine months from 743 embryo transfers, using a medium containing a high concentration of hyaluronan, were compared with data obtained during the same months one year earlier from 471 embryo transfers using a medium with a low concentration of hyaluronan. The same staff, equipment, culture medium and methods were used during both time periods. All embryo transfers were performed on Day 3. Data were analyzed with a Fisher’s exact test. All significance tests were two-tailed and conducted at the 0.05 significance level.

Results Both groups were similar on baseline variables. The clinical pregnancy rate (CPR), the implantation rate (IR) and the delivery rate (DR) per embryo transfer were all increased significantly with the use of a high concentration of hyaluronan in the transfer medium (CPR, 59.9 % vs 54.1; p = 0.04, OR 1.3, 95 %-CI: 1.0–1.6; IR, 38.8 % vs 34.2%; p = 0.02, OR 1.2, 95 %-CI: 1.0–1.4; DR, 50.1% vs 43.4 %; p = 0.02, OR 1.3, 95 %-CI: 1.0–1.7). The number needed to treat to obtain one extra delivery with high concentrations of hyaluronan in the transfer medium was 15.

Conclusion In conclusion, transfer medium containing increased concentrations of hyaluronan can significantly improve the clinical pregnancy, implantation and delivery rates after day 3 fresh embryo transfer.
The objective of this study was to determine the incidence of thrombophilic factors and its relation to IVF and embryo transfer failure. It proposed, that anticoagulant therapy renders a possible positive influence on embryo implantation. In this study we investigated IVF efficiency in the presence of anticoagulant therapy for women with thrombophilia.

Materials and Methods

The first group included 60 infertile women with tubal factor, from 1 to 5 IVF-failures previously and at least one inherited or acquired thrombophilic factor (mutation of factor V Leiden, prothrombin, methylentetrahydrofolate reductase, fibrinogen, inhibitor activator plasminogen-I, lupus anticoagulant, antiphospholipid antibodies). The second group comprised 10 infertile women with tubal factor, previously from 1 to 5 IVF-failures and without any thrombophilia. Female age was 25–42 years. The standard gonadotropin-releasing hormone agonist long protocol was used for ovarian stimulation. Women from the first group were treated by prophylactic low molecular weight heparin (LMWH) therapy (Nadroparin calcium, 2 850 anti-Xa IU/daily), from the first day of long protocol till 12th gestational week of pregnancy. LMWH therapy did not realise at the time of day and d oocyte retrieval.

Results

In the first group the prevalence of heterozygous methylentetrahydrofolate reductase C677T mutation was 53.8%, homozygous PAI-1 – 30.7%, heterozygous PAI-1 – 23%, homozygous fibrinogen mutation – 7.6%. 23% of women were positive for anti- phospholipid antibodies, more frequently for anticardiolipin antibody, B2-glycoprotein antibody. In the first group implantation rates after treatment by LMWH were 30%. First trimester abortion rates were 10%. Implantation rates in the control group (without prophylactic LMWH therapy) were 20%.

Conclusion

Thrombophilia plays a significant role in IVF-embryo transfer implantation failure. Women with repeated IVF-embryo transfer failure must be examined for thrombophilia. The prothrombotic lyses of LMWH in IVF cycle can increase the implantation rates and decrease adverse pregnancy outcome. The study will be continued.

M-039 ART clinical

Cleavage speed as an indicator of embryo implantation in ICSI cycles: A clinical evaluation from time-lapse cinematography

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Aims

Time-lapse cinematography (TLC) appears to be a useful technology in observation of the development of early human embryos in vitro. Although daily observation-based studies demonstrated that fast cleaving embryos were associated with pregnancy rate, there have been no studies that show the precise data of cleavage speed of implanted or non-implanted embryos. We put to use a built-in microscope incubator which can provide time-lapse movies from up to 90 embryos simultaneously. The purpose of this study was to evaluate the cleavage speed of human embryos in association with outcome of single embryo transfer.

Methods

We conducted a matched case-control analysis from 27 pairs of implanted and non-implanted ICSI and fresh single embryo transfer cycles between January and September in 2009. In the period, all the fertilized oocytes were observed using the TLC incubator. The images of each embryo’s development were recorded at the concave bottom of a microwell recorded by taking photographs every 10 minutes in the TLC incubator. Single embryo for transfer was selected on the basis of conventional embryo grading system from the TLC images. Each case was matched to a control by age, duration of infertility, and number of previous IVF or ICSI treatment cycle. Data are expressed as the mean ± SD. Significant differences were determined using Student’s t-test when appropriate. Statistical significance was defined as p < 0.05.

Results

In an implanted and a non-implanted group, time from ICSI to pronuclear disappearance, time to 2 cells, time to 3 cells, time to 4 cells, and time to commencement of morula formation was 1.04 ± 0.08 day and 1.14 ± 0.12 day (p = 0.009), 1.14 ± 0.08 day and 1.25 ± 0.13 day (p = 0.006), 1.67 ± 0.12 day and 1.75 ± 0.21 day (p = 0.024), 1.73 ± 0.18 day and 1.85 ± 0.22 day (p = 0.058), and 3.45 ± 0.47 days and 3.32 ± 0.33 days (p = 0.28), respectively.

Conclusions

In the development of ICSI-treated embryos, fast pronuclear disappearance and cleavage speed of earlier stages may be the predictive information. However, we failed to show that early beginning of morula formation is a valuable criterion in the selection of the most embryo for transfer.

Source of Funding of the Current Study

none.

M-040 ART clinical

Single embryo transfer using built-in microscope time-lapse incubator

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Aim

The brand-new built-in microscope incubator allows time-lapse observation of up to 90 embryos at the different site. Therefore, we can select good or poor quality embryos from the movies without opening the incubation door. We hypothesized that such full-scale time-lapse observation can promote elective single embryo transfer (eSET), which may reduce multiple pregnancy rates.

Methods

We applied the built-in microscope incubator system for the consecutive 289 fresh embryo cycles that include 1,289 embryos. 8 cycles (22 embryos), in which cleaved embryos were not available, were excluded from the current study. Patient age distribution is as follows: 27 cycles for 25-29 yrs, 90 cycles for 30–34 yrs, 105 cycles for 35–39 yrs, 34 cycles for 40–44 yrs, 3 cycles for 45–49 yrs. Various protocols were included such as GnRH agonist short protocol, GnRH antagonist protocol, clomiphene citrate cycle, and natural cycle. Single step medium was used.

Results

Fresh embryo transfer was done in 259 cycles, except 23 cycles (118 embryos) including 15 all freezing cycles. Day 2, 3, 4, 5, and 6 transfer was done in 54, 97, 61, 45, and 2 cycles, respectively. 15 double embryo transfers were included in our early series. Patients could readily accept which embryo had grown best, using the data from time-lapse video. Thus SET was done in 244 cycles (94.2%), including 49, 89, 58, and 45 cycles of Day 2, 3, 4, and 5 SET. Overall pregnancy rate (PR) was 32.4%. Fetal heart beat was confirmed in 62 cycles (24.1%). Monzygotic twinning was observed in one case (Multiple pregnancy and delivery rate: 1.6%). Day 4 transfer was powerful in our system (PR 39.3%). Live birth delivery rate of SET was 29.6%, 33.0%, 18.3%, and 9.1% at the ages of 25–29, 30–34, 35–39, and 40–44, respectively.

Conclusion

Time-lapse images were useful in understanding of cleavage, compaction, and blastocyst formation. eSET was promoted since the information from TLC is readily acceptable to the patients in addition
to the medical staffs. Our novel system promoted eSET without sacrificing PR. Further studies are required using our new built-in microscope incubator.

Source of Funding of the Current Study none.

M-041 ART complications
Postnatal morbidity and mortality increased to ART
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Background There has been a substantial increase in the demand for assisted reproduction technology (ART) in aged women.

Statement of purpose Determining relation of increased morbidity and mortality in the postnatal period.

Statement of method We have performed a review on state of the art problem in a worldwide basis and our own experience. Search strategies included online searching of the MEDLINE database and hand searching of relevant publications and reviews.

Statement of results The use of assisted reproductive technology accounts for a disproportionate number of low-birth-weight and very-low-birth-weight infants. Low birth weight infants have an increased risk of developing cardiovascular and coronary heart disease, hypertension, diabetes and stroke in adulthood. There is increasing evidence that the manipulation of gametes and embryos as practised in human IVF involve perturbations in foetal and neonatal phenotype. The increasing use of ICSI introduces additional variables and attendant risk. Several studies have indicated that epigenetic programming may be disrupted after ART. Epigenetic regulation of gene expression is critical during spermatogenesis. The significance of for mental disease is becoming increasingly clear.

Conclusions It is expressed with insistence to acknowledge some rules in regards to ART in the same way that there are rules for the adoption process. In this, anybody is not in the same as good as necessary conditions for. Age is an issue for ART. ICSI could not be identical to ART in the same way that there are rules to acknowledge some rules in regards to ART.

M-043 ART complications
Use of Cabergolin for prevention of ovarian hyperstimulation syndrome
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Introduction The ovarian hyperstimulation syndrome (OHSS) is common complication of gonadotropin stimulation in cycles of assisted reproductive technology (ART). OHSS is caused by increased vascular permeability (VP) through ovarian hypersecretion of vascular endothelial growth factor (VEGF). Dopamin agonist Cabergolin, through its D2 receptor, is able to decrease vascular permeability and clinical manifestation of OHSS. The aim of this study is to evaluate effectiveness of Cabergolin to prevent moderate/severe OHSS in high risk patients submitted to ART treatments.

Methods We dispense 0.5 mg/day Cabergolin (Dostinex tbl) during 3 weeks starting from hCG administration in group of 37 ART patients who showed hyperstimulation risk (more than 20 follicles bigger 12 mm and/or estradiol level upper 4000 pg/ml) during practice standard long protocol with GnRH-agonist (starting in midluteal phase of preceding cycle) and recombinant FSH.

Results In the group of Cabergolin moderate OHSS developed in 8.1% (3/37), cases of severe OHSS were absent. Comparison of these results with average statistical scientific data on occurrence of moderate/severe OHSS in ART cycles (usually 15–30%) demonstrates that Cabergolin is able to decrease in 2–4 times rate of this complication. All patients received Cabergolin had the successful embriotransfer and the consecutive pregnancy were detected in 40.5% (15/37).

Conclusion Our data confirm ability Cabergolin to prevent moderate/severe OHSS without influencing the success embriotransfer in ART cycles.

M-044 ART complications
Presentation of a rare case of monoamniotic pregnancy with cord entanglement after ICSI with ovum donation – a case report
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Objectives Monoamniotic/Monochorionic twin gestation after ICSI/ET 1 in 10,000, here we present a rare case with the live birth at 36th week.

Methods A case report: Mrs. K, 38 years, with primary infertility of 10 years with endometriosis, GR IV, had laparoscopy twice earlier. Also, she had endometriotic cyst aspiration prior to IVF.

She underwent ART with ovum donation at Rao Hospital, failed in 1st cycle; in 2nd cycle of ICSI with OD 3 embryos were transferred. Conceived twins. Monoamniotic twin gestation with cord entanglement was diagnosed in the 16th week and followed with serial USG with Doppler which showed no signs of TTTS or IUGR. She was hospitalized at 32 weeks and monitored with NST, AFI and Doppler study to rule out fetal asphyxia; delivered by LSCS at the end of 35th week. Helathy twin girls were born weighing 2.2 and 2.0 kg and single placenta with 2 umbilical cords inserted close to each other, cord entanglement and true knot was confirmed after LSCS. Post natal period eventful and mother and children were discharged after 1 week.

Discussion Primary goal of USG in twin gestation is determination of chorionicity, because it has cumulative loss rate of 3% for Dichorionic and 14.5% for Monochorionic. MO-MO twins have increased risk for the following-Cord entanglement, TTTS/IUGR/ Prematurity, chromosomal anomalies, perinatal, neonatal complications and neonatal ICU care; requires intense fetal surveillance beginning at viability and ending in delivery. Elective preterm LSCS is mandatory to eliminate uncertain risk of fetal death.

Conclusion A rare case of Monoamniotic twin gestation with cord entanglement and true knot after ICSI with ET has been managed successfully at our ART center resulting in the delivery of healthy twins which did not require intense NICU management. We owe our success to the efficient team work.
M-045 ART complications
Management of ovarian torsion in vitro fertilization pregnancy
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Ovarian torsion is an uncommon cause of surgical emergency in pregnancy with an occurrence of 1 in 5000 pregnancies. The incidence of ovarian torsion in vitro fertilization (IVF) pregnancies ranges from 0.08% to 0.13%.

The occurrence of ovarian torsion in ovarian cysts in pregnancy ranges from 1% to 1.5%. The risk of ovarian torsion associated with OHSS increases if patients become pregnant subsequently. Adnexal masses with sizes between 6 and 8 cm have a higher risk of torsion, at an odds ratio of 2.8, especially between 10th and 17th week of gestation.

We report a series of 4 cases of ovarian torsion in IVF pregnancies, ranging from 6th to 13th week of gestation. All presented with iliac fossa pain of the affected side. Ovarian size ranges from 7.5 to 11.3 cm on ultrasound. Color Doppler is helpful in these cases by demonstrating lack of vascularity in the affected ovary. 2 patients experienced moderated OHSS before the index event. 1 patient underwent diagnostic laparoscopy with the affected ovary appearing necrotic after untwisting. Decision was made for open salpingo-oophorectomy. 3 other patients underwent laparoscopic untwisting ± cystectomy with successful conservation of the ovaries.

Early recognition and prompt intervention are essential for salvage of the affected ovary. Laparoscopic technique is an effective modality of treatment with lesser morbidities to the patient.

M-046 ART outcome
Looking down stream: Follow-up of the health and development of IVF conceived young adults
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Aims The first generation of young people conceived with in-vitro fertilisation (IVF) has reached adulthood. To date however, little is known about their wellbeing. The potential that preconception and intrauterine exposures associated with IVF may influence the health and development of IVF-conceived (IVFC) people warrants follow-up.

This study aims to compare the health and development of IVFC and spontaneously conceived (SC) young adults. By consulting with consumers and collaborating with a multidisciplinary team, strategies have been developed to overcome the challenges posed by follow-up decades after IVF treatment.

Methods By 1992, over 1200 singleton children had been born as a result of IVF at 2 centers in Melbourne, Australia. Mothers of these children are traced using health services, Australian Electoral Commission and National Death Index records. When a current address is available, the IVF center sends a registered mail letter of invitation to participate in the study. Women who consent are then contacted by the independent research team and complete a computer assisted telephone interview (CATI) about their offspring’s health and development from birth to the present. The mothers are also asked if they consent to the researcher contacting their son/daughter. Where permission is given the young adult is contacted and those who agree complete a CATI about their physical, mental, sexual and reproductive health, education, and occupation. The interview includes standardised measures used in other Australian studies of young adults and study-specific questions. Data will also be collected from a SC comparison group.

Results To date, over 390 IVF mothers have been approached, 66% have responded and of those participating, 81% have given permission for their offspring to be contacted. Almost all (99%) of the young adults approached have agreed to take part. So far, 190 IVF mothers and 125 young adults have been interviewed with data collection to be finalised in 2012.

Conclusions The inherent difficulties in contacting people 18–28 years after IVF treatment can be overcome. We are confident that our tracing and recruitment strategies will allow us to successfully follow-up a sufficiently large sample of IVFC young adults to ascertain their health and development. Funded by the National Health and Medical Research Council.

M-047 ART outcome
Successful neonatal outcome after transfer in day 3, day 5 and day 6 of vitrified human embryos
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Aims Recent clinical reports not only show that vitrification of oocytes can be successfully used for human fertility treatment, but also vitrification of embryos and blastocyst may be a routine procedure to human assisted reproductive technologies. Vitrification appears to be associated with a significantly higher post thawing survival rate than slow freezing. Our aim was to evaluate the neonatal outcome of vitrified embryo and blastocyst transfer of supernumerary embryos which were vitrified and transferred after warming.

Methods Analysis of 44 women who delivered 50 babies from thawed vitrified embryos and blastocysts. We evaluated 124 cycles of frozen embryo transfer since January 2007 to February 2009. We thawed Day 3, Day 5 and Day 6 embryos and transfer them in Day 3, Day 5 and 6. The embryos in Day 3, Day 5 or Day 6 were vitrified using ethylene glycol and Propanediol by cryotop method and transferred after warming, the same day or two or three days later, after warming and culture in blastocyst stage. We analyzed: post-thaw survival rate of embryos, implantation and pregnancy rate, neonatal outcome, and congenital birth defects.

Results A total of 231 embryos vitrified in Day 3, Day 5 or Day 6 embryos from 112 cycles were warmed and 207 (89.6%) of them survived, which were used for embryo transfer. The pregnancy, implantation, miscarriage, and live birth rates, were as follow: 50 pregnancies (44.64%), 56 sacs (27.05%), 6 abortions (10.71%), and 50 Newborns (89.2%) respectively. Of the 44 deliveries: 38 pregnancies (86.36%) were singles, 6 (13.6%) were twins, and 0 % were triplets. 5 deliveries (11.36%) were preterm. The mean APGR was 8/9. No congenital birth defects were reported. The newborn weight in average was 2400 grs. 29 babies (58 %) were females and 21 (42%) were males.

Conclusions This preliminary study shows that vitrification is an efficient method of cryopreservation of human day 3, day 5 or 6 embryos with neonatal outcome comparable to fresh embryo transfer. The vitrification of embryos and blastocysts is a simple, easy, and quick method. This technique achieves the same high pregnancy and implantation rates as fresh embryo and blastocyst transfer. Live birth rates in this study were similar to fresh embryo and blastocyst transfer, proving the safety of this method. More trials are necessary to reconfirm these results.

M-048 ART outcome
Weekday oocyte retrieval vs weekend oocyte retrieval: Do weekend ICSI cycles have an impact on the outcomes?
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Introduction Despite attempts to program intracytoplasmic sperm injection (ICSI) cycles with oral contraceptive pills and...
GnRH agonists, ovarian stimulation is not always predictable, causing some oocyte retrievals to occur on weekends. Whether working during the weekend may be a cause of stress and tiredness, leading to the impairment of ICSI outcomes is still to be elucidated. The aim of this study was to evaluate if ICSI outcomes are influenced by the day of the oocyte retrieval.

Materials and Methods ICSI cycles whose retrievals were performed on Wednesdays and Sundays were analyzed. Patients were subdivided into two groups according to the day of oocyte retrieval: Wednesday (group A, n = 196); Sunday (group B, n = 131). Groups were compared regarding ICSI outcomes. The data are expressed as mean ± SD. A p-value < 0.05 was considered as statistically significant.

Results There were no significant differences regarding cycle’s general characteristics. Similar female age (34.5 ± 4.8 vs 34.3 ± 4.9; p = 0.8079), total dose of recombinant follicle stimulating hormone (FSH) administered (2306 ± 699 vs 2258 ± 813; p = 0.2808), estradiol (E2) levels on the day of hCG administration (1883 ± 1847 vs 1985 ± 2183; p = 0.8565), number of aspirated follicles (17.6 ± 13.5 vs 16.7 ± 12.9; p = 0.5328), oocyte yield (67.5% ± 62.9%; p = 0.1026) and metaphase II (MII) oocyte rate (71.7% vs 69.1%; p = 0.3589) were observed. Furthermore, no significant differences between groups A and B regarding fertilization rate (68.9% ± 72.5%; p = 0.1598), implantation rate (21.8% vs 24.3%; p = 0.5714) and pregnancy rate (29.9% vs 31.6%; p = 0.7129) were found between the groups.

Conclusion ICSI performance’s day does not seem to interfere on ICSI outcomes. No significant differences were found between groups A and B regarding all the evaluated variables. Indeed, there was a slightly trend towards better results in patients who underwent weekend cycles. This finding could possibly be explained by the fewer number of patients programmed to undergo oocyte retrievals on the same weekend day, and due to the fact that staff scheduling could not only overwhelm their tiredness and stress, but also reflect in fewer nurses, physicians and embryologists involved in the in vitro fertilization procedures. A well trained embryologist’s group adhered to staff scheduling allows large programs to ensure a similar outcome regardless of the day in which embryologists perform the gametes manipulation.

Funding none.

M-049 ART outcome Social consequences of the reproductive law – review of different models

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Legislation for assisted reproductive techniques (ART) may have fundamental consequences due to ethical, social, and economic impact of such formal statutory regulations. Infertility treatment with ART has been governed by law in some European countries since 1990s, however in contrast in other parts of the world (e.g. USA and Japan) there is little statutory regulation and ART policy relies on self-determined guidelines. Despite such regulatory diversity there is a common belief, especially in Europe, that ART requires stronger legislative control. Majority of European countries have some form of statutory governance, with only few (e.g. Poland and Ireland) missing formal legislation.

Restrictive regulations (such as Italian Law 40/2004 and Germany’s ART laws) assume to protect the embryo, and totally ban cryopreservation as well as genetic analysis of the embryos. These conservative regulations limit the number of fertilized eggs to 3 (or even to 2 in Polish conservative legislative proposal) and impose mandatory transfer of all embryos obtained. This approach (as documented by Italian experience and numerous reproductive models) clearly lowers pregnancy rates. It also markedly increases rates of multiple pregnancies, including triplets, that are associated with higher maternal morbidity and mortality as well as higher perinatal complications. Consequently restrictive ART laws results in higher costs of achieving a pregnancy and higher costs of neonatal care.

In some European countries (e.g. Belgium and Sweden) elective single embryo transfer (SET) is an ART policy either by law or by guideline. Elective SET in fresh IVF cycle yields lower pregnancy rates than double embryo transfer (DET); embryo transfer policy adopted in majority of European countries, e.g. UK), but simultaneously SET lowers rates of multiple gestations. However, as demonstrated in randomized and observational studies, the combination of SET with subsequent transfer of a single frozen-thawed embryo gives cumulative live birth rates comparable to DET. Introduction of SET with good cryopreservation programs and high quality laboratories seems to be the future of ART.

National legal regulations should remain in concordance with availability and reimbursement policy for ART. Legal restraints may lower ART efficiency with corresponding increase in cost and complication rates. Statutory regulations should guarantee equal and universal access to efficacious and safe infertility treatments.

M-050 ART outcome Outcome of assisted reproduction in women with PCOD: A prospective cohort clinical trial

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Introduction There has been a significant controversy in the literature about the outcome of assisted reproduction in women with PCOD. Some suggested a detrimental effect of the diagnosis of PCOD on the outcome of assisted reproduction (lower implantation rates and higher pregnancy loss) while others did not find such negative effect. Most of the data in the literature suffered from the two problems of retrospective design and failure to choose an adequate control group.

Methods In the current study we prospectively studied the outcome of assisted reproduction in women with PCOD compared to a control group of women without PCOD. The study group (group 1) included 122 women with PCOD while the control group included 91 women with tubal infertility (group 2). All women received oral contraceptives pills for 21–35 days in the preceding cycle followed by controlled ovarian hyperstimulation. Women with PCOD (group 1) received metformin (1000–1500 mg) one month before stimulation and continued through ART treatment. Group 1 patients were stimulated with rFSH with GnRH antagonist while group 2 patients were stimulated with rFSH or mixed protocol with GnRH agonist or GnRH antagonist. All patients underwent intracytoplasmic sperm injection. Luteal phase progesterone support was similar in both groups.

Results There was no significant difference in duration of infertility, day 3 FSH and LH level, total number of treatment days and E2 level on HCG day between the two groups. The mean age, total dose of gonadotropin, and endometrial thickness on HCG day of the diagnosis of PCOD on the outcome of ART. There has been a significant increase in cost and complication rates. Statutory regulations should remain in concordance with availability and reimbursement policy for ART. Legal restraints may lower ART efficiency with corresponding increase in cost and complication rates. Statutory regulations should guarantee equal and universal access to efficacious and safe infertility treatments.
The objective was to evaluate whether extending the embryo culture period from 2 to 3 days would yield a more optimal selection of viable embryos, thereby increasing the pregnancy rate.

Materials and Methods We have retrospectively analyzed pregnancy rates in the patients who had embryo transfer either on day 2 (382 patients) or on day 3 (387 patients) post-insemination over a 10-month period.

Results The demographic and clinical characteristics were similar in both groups. Embryos transferred on day 2 or day 3, were similar morphologically, we also find no difference in the distribution of grades between patients who became pregnant and those who failed to become pregnant. Pregnancy rates were slightly higher in patients who had embryo transfer on day 3 (40.72%) than in those patients who had their embryos transferred on day 2 (38.96%), but this difference was not significant.

Proportion of embryos with 2–3 cells, 4 cells, and 5–7 cells, which selected for replacement, showed significant difference between day 2 and day 3 (p < 0.05). There was also significant difference between pregnant and non-pregnant women based on embryo cell numbers on day 2 (p < 0.011).

Conclusion Extending the embryo culture period from 2 to 3 days had no adverse effect on pregnancy rate. Embryo transfer could be done on days 2 or 3 according to the convenience of the patient and the medical team.
weeks gestational age). Parameters examined included: Day 2, 3, or 5 embryo transfer; fresh versus frozen embryo transfer; the method of insemination, (standard IVF versus ICSI). Maternal age at the time of transfer and the gender of the offspring were also considered. Statistical analysis was performed using STATA and p < 0.05 was considered statistically significant.

**Results** Children born from frozen embryo transfers resulted in a significantly higher birth weight compared to fresh embryo transfers with mean birth weight of 3375 grams and 3254 g respectively; a 121.1 g difference, (CI: 94.3–148; p < 0.0001). There was no significant difference in gestational age between the fresh and frozen groups. There was no significant difference in birth weight between ICSI and standard IVF cycles. Furthermore the duration of culture prior to embryo transfer on Day 2, 3 or 5 had no impact on birth weight. Interestingly, male offspring were 163.4 g heavier than females (CI: 146.0–185.2; p < 0.0001). Maternal age < 40 and ≥ 40 years at the time of transfer had no impact on birth weight.

**Conclusion** We conclude that whether embryos are transferred fresh or frozen and the gender of offspring both have an independent and significant influence on birth weight. Despite the additional manipulation involved in frozen embryo transfer cycles, the resultant children have a higher birth weight than following fresh transfer. The duration of embryo culture and the method of insemination (ICSI vs standard) had no impact. These data suggest that the lower birth weight outcomes in fresh cycles are consequent upon impaired functionality of the stimulated endometrium.

**M-056 ART outcome**

**Assisted reproductive technology in Taiwan: Analysis of ART registry from 1998 to 2003**

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**Objective** To analyze the procedures and outcomes of assisted reproductive technology (ART) registry in Taiwan from October 1998 to February 2003.

**Design** Retrospective cohort study.

**Setting** 66 ART centers in Taiwan.

**Participants** Patients undergoing ART treatment in Taiwan during October 1998 to February 2003.

**Methods** Data were collected electronically by using the ART registry database of the ART procedures performed from October 1998 to February 2003 in sixty-six programs, submitted to the Bureau of Health Promotion, Department of Health, The Executive Yuan, Taiwan.

**Results** 35,017 cycles of ART treatment were registered from 1998 to 2003. Of these, 30,238 cycles involved in vitro fertilization/embryo transfer (IVF/ET), with a clinical pregnancy rate 32.6% and delivery rate 19.62% per started cycle; 187 were cycles of gamete intrafallopian transfer (GIFT), with a clinical pregnancy rate 31.0% and delivery rate 21.92% per started cycle; 3,900 were cycles of zygote intrafallopian transfer (ZIFT), with a clinical pregnancy rate 41.6% and delivery rate 29.41% per started cycle. The multiple birth rate per delivery for IVF/ET, GIFT and ZIFT were 41.09%, 51.2% and 42.93% respectively. The mean maternal age (range) in IVF/ET, GIFT and ZIFT cycles were 31 (28–45), 33 (28–45) and 33 (28–45) years old, respectively. Micromanipulation (ICSI, assisted hatching) were proceeded more than 60%. The pregnancy rate and birth rate declined with maternal age, especially after 40 years old. As a result of all procedures, 7,230 deliveries were reported, resulting in the birth of 10,416 neonates. The major malformation rate were 2.95% (103/3488) for IVF/ET singleton and 6.69% (337/5035) for IVF/ET multiple birth. The severity of ovary hyper stimulation was assessed as none, mild, moderate, and severe. After adjusting for maternal age, infertility diagnosis, fetal reduction status, the moderate or severe severity of ovary hyper stimulation syndrome (OHSS) showed a mild risk for major malformation [OR 1.79; 95%-CI: 0.97–3.31] compared with none or mild OHSS cases in the IVF/ET singletons.

**Conclusion** The use of IVF/ET is getting more popular than the other ART procedures in Taiwan. Pregnancy and live birth rates were comparable to those of other countries published.

**M-057 ART outcome**

**Blastocyst transfer of cryopreserved-thawed embryos in natural versus hormonally controlled cycles**

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**Introduction** Both natural and hormonally controlled cycles have been used during frozen-thawed embryo transfer (FET). This retrospective study compares the implantation rate and pregnancy outcome after blastocyst transfer of cryopreserved-thawed embryos in natural versus hormonally controlled cycles.

**Methods** All FET cycles (excluding donor cycles) of ovariary patients (267) done in the period between May 2000 and June 2007 were included in this study. FET was done in natural cycles in 111 patients (group 1) and with hormonally controlled cycles (GnRH antagonist, estrogen and progesterone in oil, IM) in 156 patients (group 2). All embryos were transferred at the blastocyst stage, 7 days after spontaneous LH surge in natural cycles and 5 days after the start of progesterone in hor- monal cycles. The transferred blastocysts resulted either from frozen-thawed blastocysts or from frozen-thawed zygotes that were cul-
tured to the blastocyst stage. There was no change in the freezing or thawing protocols during the period of the study. All transfer cycles were performed by the same physician under transabdominal ultrasound guidance.

**Results**

There was no significant difference between the 2 groups regarding age, duration or type of infertility, underlying etiology, quality of blastocyst transferred, or endometrial thickness on day of LH surge (group 1) or on day of starting progesterone (group 2). There was statistically significant lower number of zygotes thawed, zygotes survived, blastocysts formed or transferred from frozen-thawed zygotes and number of blasto cyst transferred (1.2 + 1.0 and 1.6 + 1.1) in group 1 compared to group 2 (p-value < 0.01, < 0.001, < 0.01, 0.001 and < 0.01 respectively). There was no significant difference in the cancellation rates between the 2 groups, (41% in group 1 and 32% in group 2). There was no significant difference in implantation rate, pregnancy rate per transfer, or in miscarriage rate between the 2 groups (29.3%, 40.9% and 22.2% in group 1 and 27%, 46.7% and 13.7% in group 2).

**Conclusion**

The data suggest that in ovulatory patients undergoing blastocyst FET similar implantation and pregnancy outcome may be achieved in both natural and hormonally controlled cycles. Natural cycle has the advantage of its simplicity, cost effectiveness and ease for the patients.

M-058 ART outcome

**Specialities of delivery after ART**

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Since the first child was born after fertility treatment 30 years ago, the number of assisted pregnancies has increased steadily. Much interest has been put into the efficacy of assisted reproduction technology (ART) but more and more research now focuses on the safety of delivery.

In European countries up to 4% of all deliveries now result from fertility treatment, in Russia the rate of delivery after ART is less then 1% (2007). Compared with fertile women, women who conceived by IVF had an increased risk of complications of pregnancy and delivery.

Our study is focused on the specialties of delivery after ART.

All women pregnant after ART booking for delivery at the Obstetrics Department of Research Institute of Maternal and Child Care in 2006 were included in this study. The rate of delivery after ART consist 3.8% (98) of all deliveries. Women with a singleton pregnancy consist 65.3%, women with multiple pregnancy 34.7%. Vaginal delivery was registering in 20.4% (20 cases), including 17 singleton pregnancies and 3 twins. Planned cesarean section was made in 67.3% and emergency cesarean in 12.3%. The main reason for planned caesarean included maternal age more than 35 years and repeatedly tries of ART in the past (37.8%), not head-down position (21.2%), preeclampsia (21.2%), multiple pregnancy three fetus (12.1%), uterine scar (7.6%), placenta previa (3%). Main medical reasons for emergency cesarean were placenta abruptio (41.6%), fetal distress (25%), failure of labor to progress (25%) , prolapsed umbilical cord (8.3%).

In conclusion, we found the increase of vaginal delivery after ART compared with 2002, when abdominal delivery consisted 98%. Delivery after ART is needed to ensure the mother’s and infant’s well-being, but not only by cesarean section.

The study was funded by the State.

M-059 ART outcome

**A comparative analysis of cytokines in the follicular fluid of patient with various outcomes of in vitro fertilization**

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The search for markers suitable for the prognostic of the outcome of in vitro fertilization (IVF) so far has brought no success. The evidence on follicular fluid (FF) cytokines as candidates for these markers is contradictory. We have analyzed the quantity of IL4, IL18, IFNα and IFNγ in FF of infertile females (n = 70) with positive pregnancy, n = 21, group 1) or negative (no pregnancy, n = 49, group 2) outcome of IVF. IFNγ was identified in concentrations varying from 2.4 to 15.5 pg/ml (mean value 9.4 ± 4.5) in 5 out of 60 patients 70 (7%). Pregnancy was not achieved in any of them. IFNα concentration varied from 0.4 to 11.4 pg/ml (mean value 1.33 ± 2.0) in 35 out of 70 patients (50%). In group 1 patients mean IFNα concentration was significantly lower than in group 2 patients 0.65 ± 0.67 vs 1.4 ± 2.14 (p < 0.005). IL8 and IL4 were detected in all patients, with no difference in the mean values in both groups. However, there was a statistically significant intergroup difference in the IL4/IFNα ratio (6.18 vs 3.65; p = 0.002).

In all cases with high IFNα and the presence of IFNγ in FF we observed a 3-fold increase in the activity of serum IgG against proteins of at least one causative agent of TORCH. In a patient with recurrent genital herpes IFNγ concentration in FF was 7.7 pg/ml, IFNγ concentration reached a maximum of 11.4 pg/ml, and the IL4/IFNγ ratio was critically low (0.7). IVF was unsuccessful.

From these findings it can be concluded that high concentrations of IFNα and IFNγ and low IL4/IFNα ratio in FF are negative prognostic markers of IVF outcome. High concentrations of IFNα and IFNγ correlate with activity of antibodies against infectious agents which protects from development of diseases, but are not suitable for embryos implantation.

M-060 ART outcome

**European retrospective chart review of gonadotropin use during in vitro fertilization demonstrates lower total IU usage per cycle with rFSH compared with hMG-HP**

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**Introduction**

Due to its higher unit cost, the common perception is that use of recombinant follicle stimulating hormone (rFSH) in an in vitro fertilization (IVF) cycle is more costly than a cycle using highly purified human menopausal gonadotrophin (hMG-HP). The objective of this study was to determine the average total international unit (IU) usage in IVF cycles using rFSH alone compared with those using hMG-HP alone following long GnRH agonist protocol for pituitary down-regulation.

**Materials and Methods**

This study was an observational retrospective medical chart review of anonymous databases from European patients. Patients received rFSH or hMG-HP for IVF (with or without Intracytoplasmic Sperm Injection, ICSI) across centers in Spain, Germany, Denmark and Switzerland. Anonymous data relating to gonadotrophin usage, oocyte and embryo yield, and pregnancy rates were collected from clinic records. More than 30,000 IVF cycles were included in the review. Statistical analysis of the primary efficacy variable was conducted using a 2 sided t-test.

**Results**

The number of IU of rFSH used per cycle was significantly lower than that for hMG-HP (207.3 ± 769.3 IU vs 2540.14 ± 883.08 IU; p < 0.01). The average length of gonadotropin treatment per cycle was significantly shorter with rFSH than hMG-HP (11.41 ± 2.24 days vs 11.65 ± 2.42 days; p < 0.01), and the mean starting dose of treatment was significantly lower for rFSH than hMG-HP (11.41 ± 2.24 days vs 11.65 ± 2.42 days; p < 0.01), and the mean starting dose of treatment was significantly lower for rFSH than hMG-HP (11.41 ± 2.24 days vs 11.65 ± 2.42 days; p < 0.01). However, there was a statistically significant intergroup difference in the IL4/IFNα ratio (6.18 vs 3.65; p = 0.002).

In all cases with high IFNα and the presence of IFNγ in FF we observed a 3-fold increase in the activity of serum IgG against proteins of at least one causative agent of TORCH. In a patient with recurrent genital herpes IFNγ concentration in FF was 7.7 pg/ml, IFNγ concentration reached a maximum of 11.4 pg/ml, and the IL4/IFNγ ratio was critically low (0.7). IVF was unsuccessful.

From these findings it can be concluded that high concentrations of IFNα and IFNγ and low IL4/IFNα ratio in FF are negative prognostic markers of IVF outcome. High concentrations of IFNα and IFNγ correlate with activity of antibodies against infectious agents which protects from development of diseases, but are not suitable for embryos implantation.
less IU per cycle, versus hMG-HP. These results suggest that the perceived costs of treatment with rFSH are currently over-estimated, and the higher unit cost of rFSH is potentially offset by a lower required dosage compared with hMG-HP.

This research was funded by Merck Serono.

M-061 ART outcome
Quality improvement research in a large IVF clinic and the effects of hyaluronan-containing transfer medium on delivery rate

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Introduction An important aspect of health care development is to conduct quality improvement research to allow the assessment of quality of care in a total population beyond what is possible with randomized clinical trials. The purpose with this work was to perform quality improvement research in an IVF clinical setting and evaluate the influence of a high hyaluronan-containing transfer medium on clinical pregnancy, implantation and delivery rate.

Methods Data, including all treated patients, was collected between the years 2004 and 2008 on pregnancy, implantation and delivery rate from nearly 3,000 embryo transfer cycles. At our clinic the mean age of the female patients is 31 years and we transfer between 1–3 embryos per patient (mean 2.1). The annual number of embryo transfers for the years 2004–2008 were as follows; 537, 674, 958, 1248 and 1532 respectively. During the second half of 2006, a high hyaluronan-containing transfer medium was introduced into the routine work. Data were analyzed with a Mantel-Haenszel Chi-square test and conducted at the 0.05 significance level.

Results The clinical pregnancy rate per embryo transfer for 2004, 2005, 2006, 2007 and 2008 were as follows; 56%, 56%, 56%, 64% and 61% (p = 0.00). The implantation rate for 2004, 2005, 2006, 2007 and 2008 were as follows; 37%, 36%, 37%, 43% and 41% (p = 0.00). The delivery rates per embryo transfer for 2004, 2005, 2006, 2007 and 2008 were as follows; 48%, 46%, 47%, 54% and 51% respectively (p = 0.009).

Conclusion In a large IVF clinic with more than 1,200 cycles annually a high hyaluronan containing medium significantly increases the clinical pregnancy, implantation and delivery rates. Despite the substantial increase in treatments over time it was still possible to further increase the most important outcome, delivery rate.

M-062 ART outcome
Extended embryo culture success rates, a matter of patient profile and type of treatment

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Introduction Embryo transfer routinely takes place 2 or 3 days after egg collection, whereas, blastocyst transfer places the embryo into the uterus at a later stage (Day 5 or 6) which approximates the time an embryo would normally arrive in the uterus. Therefore, the aim of this study was to characterise groups of patients that would benefit from a planned blastocyst transfer and which groups should be discouraged from pursuing such therapy.

Methods We performed a retrospective analysis of 338 patients who underwent Day 5 or day 6 transfer at our unit. SET was undertaken for all patients, however if there was no leading blastocyst on day 5 of culture, we transferred 2 embryos. Couples in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were sub-grouped according to female age: 1 = age < 35 years, 2 = 35–39 years and 3 = 40 years. The following parameters were analyzed: initial infertility diagnosis, day 3 FSH levels, fertilisation and cleavage rates, number of embryos transferred and clinical pregnancy rates.

Results We identified 226 IVF and 112 ICSI couples that had a transfer. 85 patients were identified in IVF I. Four patients had 2 embryos transferred which resulted in 3 singletons, 39 patients with one blastocyst transferred became pregnant. 119 patients were analyzed in IVF II. 7 patients had 2 embryos transferred resulting in 4 pregnancies with 1 set of twins. 44 from the one blastocyst transfer group became pregnant. A total of 22 patients were in IVF III. In this group 11 patients had 2 embryos transferred, 3 achieved a singleton pregnancy with no twins reported. 4 patients from this group who elected for a one blastocyst transfer became pregnant. In ICSI I, 51 patients were analyzed for pregnancy. Overall in this group, 5 patients had 2 embryos transferred resulting in 1 singleton pregnancy, 15 from the one blastocyst transfer group. Within ICSI II, consisting of 52 patients, 7 patients had 2 embryos transferred resulting in 2 singletons, 1 set of twins and 1 set of triplets. 15 patients with one blastocyst transferred became pregnant. ICSI III consisted of a total of 9 patients and 2 achieved a singleton pregnancy, one from the 2-embryo transfer and one from the SET group.

Conclusion When eSET extended culture is implemented pregnancy rates are highest in young IVF patients with anovulation and unexplained infertility and multiple pregnancy rates are less than 5%. This is the target group for such therapy.

M-063 Contraception
Oral contraceptive use and hypertension

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Background OCP use is the mostly reason for secondary hypertension. The effect of this agent on the renin angiotensin system and sodium metabolism is possible mechanisms of the hypertension. Plenty of use, each interference between ocp use and public health is important, thus women and health observers worry for danger of ocp use.

Materials and Methods This study was a accomplished in several health clinic in Shiraz, Iran. In this retrospective study, 165 women under 40 years old who used ocp selected and information a bout their personal and demographic particularities, obstetric history and history of ocp use extracted from their folders. Blood pressure and weight in first visit and after 6 and 12 months of ocp use were studied.

Results Results showed that difference between the average of systolic blood pressure 6 months after ocp use and first blood pressure was not significant but difference between average systolic blood pressure 12 months after ocp use and first blood pressure was significant (p = 0.03) and this difference depends on kind of ocp. This difference in low dose pills (LD) was significant rather than triphasic pills (p = 0.01). But difference between average diastolic blood pressure after 6 months and 12 months of use was not significant.

Conclusion Probably estrogen in ocp increases plasma level’s angiotensinogen nearly its level in normal pregnancy.

Because of oral contraceptive’s effective blood pressure depend on its dose and present pills that have low estrogen dose therefore they have a light effect on blood pressure but blood pressure control for identify individual response is recommended.

M-064 Contraception
Compare the efficacy of single dose vs 2 doses regimen of levonorgestrel for emergency contraception

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Background Emergency contraception is available for women up to 72 hours after unprotected intercourse. One of acceptable Emergency contraception in Iran is 0.75 mg doses taken 12 h apart of levonorgestrel
regimen. In our community are not used of single dose 1.5 mg levonorgestrel regimen.

**Objective** To compare the efficacy of single dose vs 2 doses regimen of levonorgestrel for emergency contraception.

**Methods** This study was a double blind randomized trial and conducted from March 2009–April 2009 in 5 family planning units. Questionnaire and information registering sheets were used to obtain data. 235 healthy women with regular menstrual cycles and in reproductive age (16–45 years old) who requested emergency contraception within 72 h of unprotected intercourse were participated in this study. They were randomized 2 groups (100 women in each group). A group were received 1.5 mg of levonorgestrel and B group took 2 doses (0.75 mg 12 h) of levonorgestrel.

**Results** Of 200 women with known outcome, mild side effects such as nausea, vomiting, fatigue, dizziness, headache did not differ between 2 groups (p = 0.74, p = 0.8, p = 0.67, p = 0.64, p = 0.48 respectively). Pregnancy rates were 1% (1/100) in women given single dose levonorgestrel, 2% (2/100) in those given double dose levonorgestrel. These proportions did not differ significantly (p = 0.5). The expected day of menstruated was significantly longer in double dose vs single dose of levonorgestrel (delay of menses > 7 days) (p = 0.01) most women in single dose group menstruated within 3 days of the expected date. The occurrence of breast tenderness, diarrhea and lower abdominal pain were significantly less in single group than the 2-doses-group (p = 0.02, p = 0.02, p = 0.001 respectively).

**Conclusion** These data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that these data are supportive that.

**M-066 Contraception**

**Evaluation of multi-load 375 and TCU380 A-I.U.D complications in women referred to the health and treatment settings of Kermanshah Medical University, Iran**

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**Introduction** Regarding the extensive use of IUD as contraception, the study was conducted to evaluate the complications resulting from Tcu380A IUD inserted in women referred to Health & Treatment setting of Kermanshah Medical Science University.

**Materials and Methods** In this descriptive study, 245 women using Tcu380A for at least 6 months were assessed. The tools used in this study included medical and obstetric records performed by gynecologists and obstetricians. The following variables: Pregnancy, bleeding, ectopic pregnancy, uterine perforation, and expulsion, pelvic inflammatory disease (PID) were assessed. To analyze the data descriptive statistics and Chi-square, student-t and Fisher test were used.

**Results** Severe bleeding during menstrual cycle was the most common complication in the 2 study groups, especially in the multiload 375 IUD group (75 vs 41.6% ; p < 0.001). The multiload 375 IUD had a significantly higher dysmenorrheal rate compared to the Tcu380A (70.8 vs 40.8% ; p < 0.001). The rate of interauterine pregnancy was low for the tow device (1.7 vs 2%), and occurrence of ectopic pregnancy in multiload 375 IUD was 0.8% and in Tcu380A group was not reported. There were no uterine perforation observed for either device.

**Conclusion** According to the results gained, the common complications in each group were not dangerous and their severe and threatening complications were uncommon. The most frequent complications were severe bleeding during menstrual period, dysmenorrheal and spotting and these complications were seen more among those using M.L375IUD.

**M-067 Contraception**

**Association between the common methods of contraceptive use and invasive cervical carcinoma**

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**Background** Invasive cervical carcinoma is one of the most common malignancies in women. The risk factors are old age, low socioeconomic status, multiparty, first intercourse in low age, multiparous, history of infection and sexual transmitted disease (STD) smoking, long term use of oral contraception (OCP), malnutrition, and so on. There has been a possible relationship between invasive cervical carcinoma and OCP or any other methods of contraceptive, the contraceptive methods are commonly used in I.R. Iran. It was thus aimed to study the relationship between the methods of contraceptive use and cervical carcinoma.

**Materials and Methods** In this case-control study, 109 Iranian Moslem patients with cervical carcinoma were selected. The control group (218 individuals) was Iranian Moslem patients that referred to hospital with any reason except gynecology disease, and had a normal pap-smear in the last year and had no history of colposcopy, cryotherapy, electric cauterization and conization.

**Results** Almost all (99%) of the cases and 87% of the control group had a history of contraceptive use (p = 0.0004). Of which, 71% of the cases and 67% of control group had a history of use oral contraceptive (p = 0.52), 15% and 13.5% of the respective group had positive of use IUD (p = 0.75) and 43% of the cases and 31% of the control group had a history of use of condom (p = 0.028).

**Conclusion** There was no statistically significant correlation between OCP or IUD and cervical carcinoma, but there was a significant correlation between the use of condom and cervical carcinoma. Protective effect of condom has relationship with continuously and long term use, but patients in this study no these characteristics.

**M-068 Contraception**

**Women’s educational needs about emergency contraception method**

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**Introduction and Goal** Emergency contraception (EC) method, is a way that women can use it when they did not use any sure method for prevention of pregnancy, or every case that has probability of unwanted pregnancy. They can use this method for 3 days (72 hours). The goal of this research was investigation of women’s educational needs about EC and its details.
Materials and Methods We did this re-
search (sectional-descriptive) for investi-
gation women’s educational needs about EC, in 250 women between 15 and 45 years old, that came to health centers in Boroujeerd. We use nonrandom-continuum way for select our samples. The instrument of gathering in-
formation was questionnaire, and we gath-
ered information by interview. Information was analyzed with SPSS software.

Findings The information showed that 85.3% of women have moderate knowledge about EC method, 13.6% have knowledge about all details of EC. 32.5% have knowl-
dge about EC side-effects. 85.3% have knowledge about EC contradictions, 53.6% did not have knowledge about starting time and how to use this method, 90% did not have knowledge about caring points during use of EC.

Conclusions According to results, women have needs for education about all details of EC, we must plan for them in health centers to achieve this aim, and physicians, nurses, midwives can educate women through books, pamphlets, films in this matter. Because with exact planning for having healthy women we would have healthy community, after that women and their children would be happy and healthy, too, and it is principal aim of our researches.

M-069 Contraception Enhancement of conception or contraception by a unique and novel approach
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Objectives
A) To pinpoint the time of ovulation and thus enhance conception or contraception.
B) To simplify the cervical secretion collect-
ion and increase the quantity collected, facilitating more accurate evaluation.
C) To narrow the fertile window to 3 days for conception and 7 days for contraception.

Design and Methods Women currently collect cervical secretions by subjective obser-
vation and thus may miss the fertile win-
dow. The fertile window is unique to every woman. The raw egg-white cervical secre-
tions and biphasic basal body temperature (BBT) are the main signs of ovulation. We conducted a pilot study of women collecting (BBT) are the main signs of ovulation. We conducted a pilot study of women collecting a large quantity of their cervical secretions, after the LH surge and the characteris-
tics of the preovulatory cervical secretions.

Conclusions The FemCap allowed women for the first time to collect large quantities of their cervical secretions and identify ovula-
tion and their fertile window with astonishing precision. This methodology shortened the fertile window to 3 days for conception and 7 days for contraception. This simple non-invasive and low cost method can maximize the chance of conception or contracep-
tion in healthy women.

M-070 Contraception Surface ultrastructure of human endometrium in Norplant users
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A prospective controlled trial was undertaken to investigate SEM endometrial changes in Norplant users. A premenstrual endometrial biopsy was taken from 20 fertile women re-
questing Norplant as a long term contracep-
tive method. Another endometrial biopsy was taken 2 years after Norplant insertion. The study showed quantitative and qualita-
tive changes of endometrial surface epithe-
lial cells in Norplant users. The number of the ciliated cells was reduced, the surface of the secretory cells was smooth without mi-
crovilli and pinopodia. Such ultrastructure changes mean that Norplant implants have an endometrial atrophic effect in women us-
ers.

M-071 Contraception Acceptability of various contra-
ception methods in women with valvular disease
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Objective The goal of the research was to study acceptability of modern methods of contraception for women with heart disease.

Materials and Methods 67 women with congenital and acquired heart disease were under observation during 12 months while 35 healthy women were involved in control group during the same period. For oral con-
traception 3 phase preparation Triregol (Gedeon Richter, Hungary) and as IUD-
Multiload Q 375 were applied.

Evaluation of hemostasis system included the following: definition of fibrinogen concent-
tration, APTT, Lupus anticoagulant (LA), prothrombin, SCFM, PDF, aggregation of thrombocytes, intensity of AT III, TAT, pro-
tein “C”, PAY.

Results Comparative analysis of blood gen-
eral coagulability, aggregation and functional activity of thrombocytes in women with ac-
quired valvular disease proved the develop-
ment of stable hyperactivity of hemostasis system after 6–9 months from the start of OK.

The monitoring of the hemostasis system during the entire period no significant deter-
rioration of blood coagulability of thrombo-
philic character was revealed in the group of women with congenital valvular disease.

In the group of women with congenital val-
acular disease no significant violation with thrombophilic character during 12 months was revealed.

During the process of application of IUD no reliable signs of activation of intravascular blood coagulability were found.

Conclusion Though during application of the system of OK to the women with heart diseases some specific intensification of the process of intravascular blood coagulability was stated, no case of clinical development of thrombophilic was noted. All the men-
tionioned proves the opportunity of OK accept-
ability for the women with valvular disease.

M-072 Contraception A vision for improving undergradu-
ate medical education in family planning and reproductive health
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The reproductive health of Russian adoles-
cents and young adults was characterized by high level of the sexual activity, sexually transmitted infections, unwanted pregnancy and abortions, poor awareness about preven-
tive methods of unintended pregnancy and infections [Uvarova E.V., 2006]. The aim of this study was to determine the knowledge of students of the Medical Academy about fam-
ily planning and reproductive health. This research included 149 students of 5–6th courses of the Kemerovo State Medical Academy (32 boys and 117 girls). The mean age of students was 22.2 ± 1.5 years. These students have been an anonymous question.

The results of our investigation showed that 90.6% of them have regular sexual contacts. A half of them had the first sexual experience before the age of 17. Mean age of the first sexual experience was 17.8 ± 2.4 years. 36.3% of the students have a permanent sexual partners, 8% of them had more than 10 sexual partners. The mean number of the sexual partners during their sexual life was
M-073 Contraception

Individual and family characteristics associated with the beginning of sexual life in female students from high school

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Objectives Evaluate the implicated factors about the beginning of sexual life in female student from public and private high school in the city of Caxias do Sul.

Methods Transversal study including 644 female students of public and private high schools in Caxias do Sul was performed from August to December 2008. According to the randomization criteria used, 13 high schools were selected (6 private and 7 public). Before participating of the research, the students read and signed an informed consent. We performed a questionnaire evaluating the economics and social situation, the sexual behavior, and the knowledge about contraception. The data were put in a scale and evaluated according to the SPSS program using Mann Whitney, Student-t-test and Chi-square methods.

Results The mean age of the female adolescents was 16.83 (± 0.98) years, and the mean age of beginning of sexual life was 17.11 (± 1.06) years and the ones who did not started sexual life was 16.65 (± 0.9) years (p < 0.005). The female students with sexual active life in the public schools represented 44.3% and in the private schools 38.7% of the total population evaluated (p = 0.173). The beginning of sexual life occurred, around 15 years of age (15.3 ± 1.36). Regarding familiar factors related to beginning of sexual life, the family income (p = 0.074) did not show significant difference between the groups (p > 0.05). However, the level of instruction of the father (p = 0.05) and of the mother (p < 0.001) were statistically significant. Evaluating the individual factors in relation to school, private vs public, no differences were detected between the two groups (p = 0.174).

Conclusions In relation to individual and familial factors involved with the beginning of sexual active life, only the levels of instruction of the parents (father and mother) and the age were different from the female students who did not have began yet sexual life. Therefore, the family gathering of the female students has important consequences in their sexual life.

M-074 Contraception

Knowledge regarding contraceptives in adolescents: The influence of the health care providers

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Objectives Verify the influence of the health providers regarding the information about the methods of contraception in adolescents.

Methods Transversal study including 644 female students of public and private high schools in Caxias do Sul was performed from August to December 2008. According to the randomization criteria used, 13 high schools were selected (6 private and 7 public). Before participating of the research, the students read and signed an informed consent. We performed a questionnaire evaluating the economics and social situation, the sexual behavior, and the knowledge about contraception. We evaluated the influence of the health professionals in a general as well stratified analysis according to the type of school, economical and social level, level of instruction of the parents, and being or not in use of contraceptives. The knowledge of the female students was evaluated throughout a 41 questions about the 12 available contraceptives. The knowledge of the female students who did not have began yet sexual life was 16.83 (± 0.98) years, and the construction of the father (p = 0.05) and of the mother (p < 0.001) were statistically signifi-
cant. After the statistical analysis, we didn’t find significant difference between the two groups (t = 0.174).

Conclusions The low index of correct questions demonstrates an urgent need to teach better our female students irrespective having or not a sexual active life. Also, this study points out a necessity for more information to be given for adolescents by health care providers.

M-075 Counselling

Genetic aspects of recurrent miscarriage

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Recurrent pregnancy loss is the miscarriage of 2 or 3 consecutive pregnancies in the first of early second trimester. Although approximately 10–15% of all recognized pregnancies result in miscarriage, less than 5% of women will experience two consecutive miscarriages, and only 1% experience 3 or more. In recurrent pregnancy loss, current practice often fails to make a diagnosis, as the fetal causes of pregnancy loss are usually ignored and only the maternal factors are assessed. The maternal causes are well known and include: Uterine factors, infections, autoimmune syndromes, endocrine abnormality, all immune factors and possibly hereditary thrombophilia. However, all assessments of these factors have been confounded by the presence of abnormal embryos that may themselves be incompatible with life. The fetal causes of embryo loss include structural malformations that are incompatible with life, and chromosomal aberrations. Unfortunately, no explanation is found in 50% to 70% of couples with recurrent pregnancy loss. About 5% of couples with recurrent pregnancy losses have a chromosomal abnormality and translocation is the most common inherited chromosomal abnormality. Although a parent who carries a translocation is frequently normal, their embryo may receive too much or too little genetic material. Couples with translocation or other specific chromosome defects may benefit from preimplantation genetic diagnosis. But in fact 60% or more of early miscarriages may be caused by a random chromosomal abnormality, usually a missing or duplicated chromosome. However, 89% of human recurrent miscarriages occur in the first trimester that this stage is too early to be diagnosed as normal or abnormal body structure on ultrasound. These defects included neural tube defects, cleft lip and cleft hand. These malformations are usually associated with a normal karyotype. Single gene disorders associated with recurrent miscarriage are myotonic dystrophy, factor V leiden mutation. The factor V leiden mutation is the most common genetic predisposition to thrombosis but its carrier frequency in the white population is 3–4%.
M-076 Counselling
The effect of sunlight exposure and leisure time counselling of lifestyle on menopausal women and their husbands

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Aim Menopause has many complications such as hot flashing, insomnia, depression, osteoporosis and cardiovascular diseases in men and women. According to the increasing of life expectancy, people suffer from menopausal complications for more times. Regarding to the importance of non-drugs methods that mainly focus on changing and improving the lifestyle, our aim of this study was evaluation the effect of nutrition and exercise counselling on menopausal women and their husband’s lifestyle.

Methods Our research is interventional study and we used random sampling. We started our study by 120 pairs of menopausal women and their husbands that were supported by Mohammadiyeh medical health center in Hamadan, Iran. We divided them to case and control groups randomly. At first we got pre test of all samples then had individual counselling to case group during 1 hour in their houses. After 2 months of our counselling, we got first post test of all and had second session of counselling, then 2 months later we got second post test of all samples. Data were analyzed by McNamara, paired T test, independent T test and Wilcoxon test, using SPSS (ver13).

Results According to McNamara test, comparison of sunlight exposure item in case and control women, pre test was (p = 0.79), the first post test was (p = 0.000). In exercise item in women by Chi-square test, pre test was (p = 0.62), the first post test was (p = 0/000) and the second post test was (p = 0/000) and in their husbands pre test was (p = 0/14), the first post test was (p = 0/000) and the second post test was (p = 0/000). In comparison painful sexual relation in two groups of women by Chi-square test, pre test was (p = 0/91), the first post test was (p = 0/023) and the second post test was (p = 0/000).

Conclusion Findings of this study showed that counselling has a significant effect on items of nutrition and exercise of lifestyle in the menopausal women and their husbands therefore counselling is a good manner to improve lifestyle.

M-077 Counselling
The effect of nutrition and exercise counselling on menopausal women and their husband’s lifestyle

Tiznobaik A
Hamedan University of Medical Sciences, Hamedan, Islamic Republic of Iran

Aim Menopause has many complications such as hot flashing, insomnia, depression, osteoporosis and cardiovascular diseases in men and women. According to the increasing of life expectancy, people suffer from menopausal complications for more times. Regarding to the importance of non-drugs methods that mainly focus on changing and improving the lifestyle, our aim of this study was evaluation the effect of sexual relations counselling on menopausal women and their husbands’ lifestyle.

Methods Our research is an interventional study and we used random sampling. We started our study by 120 pairs of menopausal women and their husbands that were supported by Mohammadiyeh medical health center in Hamadan, Iran. We divided them to case and control groups randomly. At first we got pre test of all samples then had individual counselling to case group during 1 hour in their houses. After 2 months of our counselling, we got first post test of all and had second cession of consoling. Then 2 months later we got second post test of all samples. Data were analyzed by paired T test, independent T test and Chi-square test, using SPSS (ver13).

Results According to Chi-square test, comparison of times of sexual relation in month in case and control groups, pre test was (p = 0/85), the first post test was (p = 0/035) and the second post test was (p = 0/000). In comparison painful sexual relation in two groups of women by Chi-square, pre test was (p = 0/91), the first post test was (p = 0/023) and the second post test was (p = 0/000).

Conclusion Findings of this study showed that counselling has a significant effect on item of sexual relations of lifestyle in the menopausal women and their husbands and counselling with pairs particularly in their houses, is a good manner for improvement of life style.

M-078 Counselling
Impact of maternal age and ART conception on infant outcomes and behaviour: Parental age and transition to parenthood Australia (PATPA) study

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Aim Older maternal age is associated with the risk of having a lower birthweight and/or preterm infant as well as multiple-birth. Similar associations are found for assisted reproductive technology (ART) conceptions. The aim of this study was to examine the effects of both older maternal age and mode of conception on neonatal and infant health, and parental report of infant temperament in early parenthood.

Methods The research was conducted through a controlled prospective observational study with recruitment in the third trimester of pregnancy. Participants were 425 first-time mothers aged between 20–47 years and 209 (49%) had conceived through ART. Three age groups (≤30 years, n = 114; 31–36 years, n = 125; ≥37 years, n = 125) were compared. At 4 months postpartum mothers completed a structured interview about their
The strategy of vitrification results in the total elimination of ice crystal formation, both within the cells being vitrified (intracellular) and in the surrounding solution (extracellular). The protocols of ultrarapid cooling allow low cells and tissues to be placed directly into the cryoprotectant and then plunged directly into liquid nitrogen. Growing body of evidence suggests that recently developed vitrification protocols result between 86–98% oocyte and blastocyst survival rate. Authors show their experience after two years of routine vitrification of blastocysts using Cryotop method with high survival and implantation rate with 140 thawed cycles.

**M-083 Cryopreservation**

**The comparison of outcome of vitrified-thawed embryos with GnRH agonists and antagonists in stimulating cycles**


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**Aims**

GnRH agonists and antagonists are the most widely used protocol of controlled ovarian stimulation cycles. But the difference of pregnancy rates in GnRH agonist and antagonist is still controversial. Cryopreservation of excess cleavage embryos plays an important role in IVF. We compared the pregnancy rates of frozen-thawed cycles with day 3 cleavage embryos using vitrification-thawed method which were retrieved from stimulation with hMG and rFSH in the long protocol or in the multiple dose antagonist protocol.

**Materials and Methods**

Between January 2008 and October 2009, a total 220 patients (252 cycles) were included in this study. Uterine factor, ovum donation, PGD, old age (≥ 42 years), and repeated IVF failure cases (≥ 4 cycles) were excluded. Controlled ovarian hyperstimulation was carried out with rFSH and hMG in GnRH agonist down regulation or GnRH antagonist. We compared the pregnancy rates of frozen-thawed cycles with 3 cleavage embryos using vitrification-thawed method which were retrieved from stimulation with hMG and rFSH in the long protocol or in the multiple dose antagonist protocol.

**Conclusion**

Even if most infertility specialists are aware of the deleterious effects of tobacco on fertility and ask their patients to quit, there is a significant heterogeneity in the infertile patients’ management according to physicians’ age, gender, or occupation, but not according to smoking status.
recipient uterus after standard estradiol/progesterone preparation. Quantitative β-hCG measurement was done at 13 days from embryo transfer. Clinical evaluation was performed using student’s t-test.

Results There were no statistical significant differences between 2 groups for ages, infertility duration, induction number, intactness (>1/2 intactness; 97.3%-98.5%) and pregnancy rate (60.6%-65.6%).

Conclusions According to our retrospective study, the cryopreservation outcome of human day 3 cleavage embryos does not show a statistical difference between a GnRH agonists and antagonists. Nevertheless, high pregnancy rate using vitrification method in embryo freezing gives reasonable promise of being successful technology.

M-084 Cryopreservation Bayesian analysis application and advantages in sperm biology researches – a practical example

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Background Freezing and storage of human male gametes is associated with a reduction in the overall semen quality and establishment of pregnancy. The aim of the current study was to investigate the advantages of Bayesian method in comparison to traditional methods which have been used before to analyze our data.

Methods Semen samples have been gotten from 40 men whose sperm had normal criteria. The samples were divided into 2 groups of 20. A part of each sample was separated without antioxidant as Control. Taurine (in concentrations of 25 mm and 50 mm) and Cysteine (5 mm and 10 mm) as antioxidants were prepared for each group and each group was freeze-thawed and evaluated between different concentrations and antioxidants with control. For traditional method we chose analysis of variance (ANOVA) and compare with control. For Bayesian method we chose Markov Chain Monte Carlo algorithm and discarding the initial 5000 iterations as burnin, we got the following results.

M-085 Cryopreservation Refrigeration does not increase apoptosis like markers in canine sperm

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Introduction It is essential a better understanding of damage to sperm during cryopreservation to improve current techniques of semen preservation and thus enhance fertilization rates after artificial insemination. Canine sperm can be preserved by cooling but its fertilization capacity decreases rapidly. It is argued that fertilization rates of chilled canine spermatozoa decrease significantly after 4 days of storage at 4°C, others argue that stays for 10 days. The aim of this study was to evaluate the effect of refrigeration on the parameters of motility, integrity of plasma membrane, mitochondrial membrane potential, translocation of phosphatidylserine (PS) and caspase 3 activity in dog sperm.

Materials and Methods The canine spermatozoa suspended in medium Tris-egg yolk were chilled to 4°C for 240 hours. Before and during cooling (at 2, 120 and 240 hrs.) motility was assessed under light microscopy, the integrity of plasma membrane with SYBR-14 combined with propidium iodide (PI), mitochondrial membrane potential through JC-1, translocation of phosphatidylserine by Annexin V-FITC combined with PI, and the activity of caspase 3 with FITC-DEVDfmk also combined with PI.

Results At 240 hours of cooling, the sperm showed decreased motility, plasma membrane integrity and mitochondrial membrane potential with respect to baseline (p < 0.01). Motility and plasma membrane integrity were positively correlated with mitochondrial membrane potential. Similarly the plasma membrane integrity was correlated positively with motility. The translocation of PS and the activity of caspase 3 did not increase during the 240 hours of refrigeration.

Conclusions The deleterious effect of refrigeration on the parameters of motility, integrity of plasma membrane, mitochondrial membrane potential spermatozoa only are present after 240 hours. Not presenting of apoptosis like markers (translocation of PS and the caspase activity) suggesting that this phenomenon would not be triggered by refrigeracion in dog sperm.

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M-086 Cryopreservation Sperm characteristics, use rate and art outcome in 1042 male patients with cancer referred for sperm cryopreservation before gonadotoxic treatment

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Background The deleterious impact of cancer therapy on male fertility has been largely described, and men are generally strongly advised to cryopreserve sperm before the onset of gonadotoxic treatment, making further ART cycles possible if needed. Here, we studied the relative influence of the type of cancer on sperm characteristics, and we report use rate and success rate in subsequent ART cycles.

Methods The results of the sperm cryopreservation program in men suffering from cancer at the University Hospital of Nantes were retrospectively analyzed over a 10-years period.

Results 1042 men were referred for sperm cryopreservation during this period (438 testicular cancer, 184 other solid cancers, 278 lymphoma and 142 other haematological malignancies). A total of 2577 ejaculates from 1009 men were studied. Mean age of these patients referred for semen cryopreservation was 28.6 ± 6.6 years, and 2.5 ± 0.8 ejaculates were frozen on average for each of them. Mean age was significantly lower in haematological malignancies and lymphoma group than in testicular cancer and solid cancer groups. Mean sperm concentration and total number per ejaculate were significantly lower in testicular cancer group than in any other groups. Total motility on fresh semen was significantly lower in the haematological malignancies and lymphoma group than in testicular cancer and solid cancer groups. Mean sperm concentration and total number per ejaculate were significantly lower in testicular cancer group than in any other groups. Total motility on fresh semen was significantly lower in the haematological malignancies group than in the other groups.

Frozen sperm was used for ART cycles in 82 patients (8.1%). A total of 112 IVF cycles were performed, leading to a 23.2% clinical pregnancy rate per cycle. Moreover, 66 IUI cycles were performed, leading to a 12% clinical pregnancy rate.

Conclusions Sperm quality in cancer patients can differ according to the type of cancer. However, frozen sperm before gonado-
In recent years there has been a trend against the use of egg yolk in cryoprotective media because of the hygienic risks associated with the use of animal products and the lack of quality standards [1]. Although a new generation of well-defined semen diluents, free of animal ingredients is available, egg yolk-containing extenders are still widely used for cryopreserving semen [2]. The goal of this study was to evaluate the protective effect of egg yolk compared with a soybean lecithin-based extender on sperm motility, membrane function and motility, vigor and percentage of intact acrosome (PIA) after incubation. Ejaculate of four bulls was divided into two samples, one diluted with egg-yolk-Tris extended (A) and other with a soybean-lecithin based extender (Andromed®) (B). Semen was frozen and stored in liquid nitrogen. Sperm motility was evaluated by computer access semen analysis (CASA), plasmatic membrane function by Hypotonic Swelling Test (HOST) and PIA, motility and vigor after incubation (37°C/3 hours). The statistical analysis was performed through the Analysis of Variance (ANOVA) and Wilcoxon test. The CASA results showed no statistical differences (p > 0.05) for extender A and B between total motility (69.5 ± 12.9; 57.7 ± 7.8, respectively), progressive motility (34.5 ± 10.2; 25.5 ± 3.1, respectively) and rapid sperm (53.3 ± 14.8; 47.2 ± 6.2, respectively). Evaluations of average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH) showed the same results for semen cryopreserved and selected post-thaw.

Materials and Methods 3 protocols were tested (n = 8): the same thawing protocols were used for the canine sperm, but the freezing and store of it differed:

- Exp. 1: Semen frozen and stored in liquid nitrogen (–197°C) and after incubation evaluation
- Exp. 2: Semen frozen at –80°C and stored in liquid nitrogen
- Exp. 3: Semen frozen and stored at –80°C.

Post-thawing sperm function was evaluated: integrity of plasma membrane (SYBR-14/PI), mitochondrial membrane potential (ΔΨMit), acrosome membrane integrity (PSA/FITC – PI) and translocation of phosphatidyl serine (Annexine V) by flow cytometry. The statistical analysis was performed using the Student t-test.

Results There were no significant differences (p > 0.05) between Exp 1, 2 and 3 with respect to: integrity of plasma membrane (37.7 ± 2.2; 26.5 ± 1.8 and 26.4 ± 2.2 respectively), ΔΨMit (33.8 ± 9.2; 29.8 ± 10.5, 26.4 ± 6.4 respectively), acrosome membrane integrity (41.3 ± 6.7; 41.2 ± 6.7, 39.6 ± 6.5 respectively) and translocation of phosphatidyl serine (0.26 ± 0.06, 0.10 ± 0.02, 0.07 ± 0.01, respectively).

Conclusion The storage of sperm in freezer at –80°C proved to preserve sperm function similarly to the traditional method of freezing, becoming an alternative:

- More cost effective
- Reduced storage space
- Less time and effort to find stored samples

Safer for the operator (burns, spills of liquid nitrogen when refilling, proper airing).

M-089 Cryopreservation

Functional evaluation of canine sperm cryopreserved and selected post-thaw

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Introduction Sperm freezing and thawing cause damage to different cellular structures of the sperm and its functions.

The production of reactive oxygen substances (ROS) is the major contributor of this damage. Production of ROS can be increased by the methods used to prepare the sperm after thawing for its used in reproductive techniques. The aim of this study was to evaluate the effect of sperm selection methods on the physiological function of canine spermatozoa post-thawing.

Materials and Methods We utilized canine frozen semen in straws of 0.5 ml at a concentration of 200 x 10^5 sperm/ml. The straws were thawed for 30 sec. at 37°C, the semen was washed in the medium Tris buffer 1:2 (v/v), and the sperm suspension was divided into 3 equal aliquots for the different sperm selection methods:

a) Washed by centrifugation (control), b) Swim-up, c) Percoll gradient. The different methods and post-effects were evaluated, by flow cytometry, ie. integrity of plasma membrane (SYBR-14/PI) and mitochondrial membrane potential (ΔΨMit/JC-1).

Results Swim-up properly maintained the viability and plasma membrane integrity (62.3 ± 11.7) and ΔΨMit (70.6 ± 8.2) compared with control (51.8 ± 4.1 and 75.3 ± 4.8 respectively). Not so, Percoll gradient with a significant decrease in viability and integrity of the plasma membrane (27.4 ± 0.73) as the ΔΨMit (9.3 ± 3.2) (p < 0.05).

Conclusion The Swim-up method is most appropriate for the sperm selection after thawing and offers a higher ability of preserving the viability and integrity of the plasma membrane and mitochondrial membrane support compared to the Percoll gradient method.

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**M-090 Economic issues**

**Out-of-pocket payment for ART in the public health sector in South Africa: How do households cope?**

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**Aims** Out-of-pocket payment (OOP) for assisted reproductive techniques (ART) is concerning, as it creates treatment barriers or heavy financial burdens for households (HH), especially in low resource settings. In South Africa ART is generally not covered by private health insurance schemes or government funding. This is the first study from South and sub-Saharan Africa which explored the impact of ART-related OOP on HH.

**Methods** The study was conducted at a tertiary referral clinic in the public health sector in South Africa, where ART is subsidized but patients have to contribute to cost of treatment. Eighty five consecutive IVF/ICSI cycles involving standard treatment regimens were prospectively evaluated. Couples were interviewed at completion of the ART cycle (“index cycle”). Data included socio-demographic characteristics, information on HH livelihood, financial coping strategies, demographic characteristics, and measures of emotional and financial stress. Costs were captured in South African Rands (1USD = 7.5R). Cost-expediency analysis was performed as a direct cost of all ART cycles in the last 12 months exceeding 40% of the average HH non-food expenditure.

**Results** The majority (80%) of participants were black Africans or of mixed ethnicity. The average HH size was 3.5 persons with a mean monthly expenditure of approximately R11,000. The mean OOP (direct cost) for the index cycle was R11,095 (USD 3503). Two thirds of couples had undergone previous ART, at the same institution or in a private service. Almost 1 in 4 couples faced catastrophic expenditure. Approximately 40% of couples struggled to pay HH bills, and some felt HH survival was threatened. Coping strategies varied and included selling assets, reducing spending on food and education, and delegating financial responsibility for existing children. Gender differences were observed in treatment-related emotional and financial stress.

**Conclusions** ART treatment created a large financial burden, aggravating or inducing financial difficulties of many HH and causing catastrophic costs in about 25% of couples. Participants engaged in a range of financial strategies, many of which have long-term impact. Our results are relevant for patient counselling and highlight problems in reproductive health funding in South Africa.

**Funding** The study received financial support from the MRC and the Faculty of Health Sciences, University of Cape Town.

**M-091 Economic issues**

**Relation between numbers of embryos to transfer, law and professional recommendations**

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**Background** The multiple pregnancy rate in Assisted Reproductive Technology (ART) cycles depends, fundamentally, on the number of embryos transferred. It is essential that patients and professionals should have good practical guidelines on the best number of embryos to be transferred in each cycle in order to obtain high pregnancy rates with minimal risk of multiple pregnancies. The purpose of this study is to analyze the impact made by the Spanish Fertility Society (SEF) guidelines on the number of embryos to be transferred, as regards the policies adopted at clinics in Spain, and the resulting financial repercussions.

**Methods** Data were collected from the ART register of the SEF and compared over three periods of time: 2002–2003, when there was no legal regulation and no SEF guidelines; 2004, when there was only legal regulation; and 2005–2006, when there was legal regulation and SEF guidelines. An estimation of financial impact was carried out.

**Results** The degree of acceptance of SEF guidelines varies according to the IVF technique employed. The guidelines have led to a reduction in multiple pregnancy rates, especially concerning triplets, using patients’ own eggs and with donor eggs. Over the three periods, and considering both own and donated egg cycles, the observed percentage of single pregnancies was 69.5% in the 2002–2003 period, 71.3% in 2004, and 74% in the 2005–2006 period. With respect to twin pregnancies, the observed percentage was 28% in 2002–2003, 27.2% in 2004, and 24.9% in 2005–2006. The observed percentage of triplet pregnancies was 2.5% in 2002–2003, 1.6% in 2004, and 1.2% in 2005–2006. The reduction in the financial cost of deliveries achieved in the years 2005–2006 ranges from EUR 890,187 to EUR 18,593,242, and the incremental cost per percentage point of multiple pregnancy avoided is EUR 2,989,613.

**Conclusion** Even without full implementation, these results validate the clinical utility of the SEF guidelines. They constitute a useful tool to reduce the incidence of the principal adverse effect of the ART cycles, namely multiple pregnancies.

**M-092 Embryo Efficiency of long-term embryo culture in IVF cycles**

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**Introduction** Strategy to increase accessibility to infertility services in Russia is coming to real. More than 90 clinics of ART are working. During last 5 years many ART laboratories were opened not only in Moscow or St.Peterburg, but in other cities of the country. The department of ART started its activity in 2006 in Ural Research Institute of Maternity and Child Care in Ekaterinburg. It is the oldest obstetrical institute in the city. The main feature of this ART laboratory is state support, according to which ART programs are free of charge for patients. In 2008 year 276 cycles of ART were made by support of the State, so it provides accessibility of ART. Some results of our work are presented.

The attention of embryologist has mainly focused on quality of embryo in order to increase pregnancy rate. In this study we research difference of the duration of embryo culture.

During 2008 year 331 cycles were made. The middle age of women consist 32.4 years old. The duration of infertility was 6.7 year. Embryo transfer was carried out in 285 cases. In 251 cases embryo transfer was made on Day 3 (group 1). The long term culture (Day 5) has been carried out in 34 cases (group 2). Number of embryo transferred in group 1 consisted 2,54 ± 0.07 and that in the group 2 –2,97 ± 0.13 (without significant difference). The percentage of complications were also equal in both groups: pregnancy losses have taken place in 4.8 ± 1.35% in group 1 and in 2.9 ± 2.82% in group 2. Multipletes were registered in 7.2 ± 1.63% in group 1 and in 8.8 ± 4.86% of the group 2. There were no cases of extra uterine pregnancy in group 2 and in group 1 it consists 1.6 ± 0.79%.

This study demonstrated the increasing of pregnancy rate after ART from 28.3 ± 2.8% at Day 3 to 35.3 ± 8.25% at Day 5 embryo transfer. This analysis shows the expedience of embryo culture to Day 5 and helps to optimize our work.

The study was funded by the State.

**M-093 Embryo IVF or ICSI? Idiopathic infertility and embryo quality in combined cycles**

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**Introduction** The choice of procedure (IVF or ICSI) is related to a number of indications out of which the type of infertility is considered of highest importance. In some cases like idiopathic infertility, mild asthenozoospermia, the first IVF/ICSI attempt is indicated for performing both – IVF or ICSI
in order to test fertilization potential of gametes. The aim of this study was to compare fertilization rate and embryo quality in couples with combined IVF/ICSI cycles.

Materials and Methods In the period January 2007–October 2008, 89 women with first attempt of ART treatment were included in this study. Totally 983 oocytes were retrieved and 908 of them were found to be appropriate for fertilization and separated randomly in 2 groups: group 1: 472 oocytes (52%) for ICSI and group 2: 436 oocytes (48%) for IVF.

The 2 groups were compared according their fertilization rate and embryo quality on Day 3.

Results

(1.) Fertilization rate: There is statistically significant difference (p < 0.0001) as regards the fertilization rate: ICSI — 71.2% (327 oocytes fertilized) and IVF — 51% (225 oocytes fertilized). 45% of the patients had better fertilization rate following ICSI procedure, while 27% showed better fertilization rate following IVF. 9% of them had equally fertilized oocytes following IVF and ICSI. In 17% of the cases no fertilization occurred following IVF and 2% following ICSI.

(2.) Embryo quality on Day 3: On Day 3 we divided the embryos according to their quality in 3 groups: 1) embryos with good quality, 2) embryos with average quality and 3) embryos with bad quality.

The results in the 2 groups were: Group 1 — following ICSI — 66 (21%) embryos with good quality, 88 (28%) — average and 160 (51%) — bad quality. Group 2 — embryos following IVF— 32 (15%) good, 43 (20%) — average and 143 (65%) — bad quality.

In the first group (ICSI) the embryos with good and average quality were significantly higher in number compared to the embryos from the second group (IVF).

There is a statistical difference in the bad quality embryos — they are in higher number in group 2 compared to group 1.

Conclusion There is a statistical difference in the fertilization rate from IVF and ICSI in couples with idiopathic infertility, undergoing first IVF/ICSI attempt of treatment. The fertilization rate is higher following ICSI compared to IVF.

There is significant difference in the embryo quality on Day 3 — the embryos following ICSI were with better quality.

M-094 Embryo
A study of temperature variation during Intracytoplasmic Sperm Injection and in vitro culture
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Introduction The objective of this study was to identify how the temperature in culture media in vitro varies during the culturing and Intracytoplasmic Sperm Injection (ICSI) process, and to determine how the different heat transfer methods used during the culturing process contribute to this variation.

Materials and Methods Open and closed heat transfer methods were used during the different phases of the culturing process. A Heracl cell 240L incubator using closed heat transfer was used during culturing. A MiniTub HT 50 heated stage using open heat transfer was used for the assessment and injection phases. A thermocouple linked to a datalogger was fixed to the base of the dishes so that it was continuously immersed in media. Temperature was measured every 30 seconds for 48 hours and repeated 6 times. For safety reasons these dishes did not contain embryos but followed a randomly selected dish containing embryos through each stage of the process.

Results When measured, the open heat transfer methods proved to be far less stable than the closed heat transfer methods with the media temperatures varying by as much as 5.6°C in the open heat transfer methods. The greatest factor affecting temperature variation was the duration of the injection process which was dependent on the number of oocytes to be injected.

Another significant factor was multiple opening and closing of the incubator to conduct embryo assessments. The significance of this factor increased with the increase in the number of dishes to be assessed in the incubator. The temperature of media within dishes varied from day to day to day when they were being assessed on heated stages, these variations corresponded to changes in ambient atmosphere inside the culture room.

Conclusions We showed that during the ICSI process and subsequent culturing, media temperature varies by as much as 5.6°C. The greatest temperature variation was found during injection.

This variation can be reduced by reducing the number of oocytes in each dish so that the time the dish spends outside the incubator is reduced to a minimum. The number of dishes stored in an incubator and the number of times the door is opened also has a significant impact on temperature variation within culture media.

Closed heat transfer systems generate significantly less variation over open systems.

The performance of heated stages varies with the ambient atmosphere inside the culture room so maintaining stable conditions in the room is critical.

M-095 Embryo
Influence of intraperitoneal transfer of peripheral blood mononuclear cells on embryos’ implantation rates in patients after infertility curing by method of IVF
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Introduction Embryo implantation in the uterus is one of the most important stages to achieve pregnancy in infertile patients after IVF cycle. The endometrial state of the patient should be regulated by the endocrine system. It is a well-known fact that human endometrium is able to accept embryos only during some limited period. So it is very important to prepare endometrium to embryo transfer to achieve embryo implantation. There is a suggestion that maternal immune cells are able to support the process of embryo implantation. So the aim of our work was to investigate and to compare pregnancy rates in infertile patients after IVF cycles using intrauterine transfer of peripheral blood mononuclear cells (PMBC) before embryo transfer.

Methods and Results 100 infertile patients were investigated. All the patients had experienced 2 or more unsuccessful IVF cycles. 100 infertile patients were divided into two groups. Both groups included 50 patients. PMBCs were not used in the first group. The middle age of the patients in the first group was 37.5 ± 3.4 years. The middle age of the patients in the second group was 39.5 ± 5.5 years. PMBCs were used for the patients of the second group. PMBCs were obtained from patients in the day of oocytes receiving. After that peripheral blood cells were cultured during 48 hours. 2 days later fresh mononuclear blood cells were obtained. The mixture of cultured and fresh obtained blood cells was transferred to uterus cavity. The embryo transfer had a place on the third or on the 5th day of embryos’ culture. In the non-treated group the clinical pregnancy rate was 20% in total (the pregnancy was achieved in 10 cases). Among those achieved pregnancies 60% (6 cases) were obtained after transfer performed on day 5. 40% of clinical pregnancies (4 cases) were obtained after the embryo transfer on a Day 3. In the second group the clinical pregnancy rates were 36% (18 cases). Eleven of those pregnancies (61.1%) were obtained after embryo transfer on a day 5. In the 7 cases (38.9%) the clinical pregnancy was obtained after embryo transfer on a day 3. In both groups 2 embryos were transferred in uterus cavity in the each case. The quality of transferred embryos was good in every case of transfer.

Conclusion Intrauterine PMBC application increases rates of embryo implantation
Aneuploid embryos are a consequence of one of the gametes being aneuploid. The consequences of oocyte aneuploidy, particularly due to maternal age, on embryo aneuploidy, implantation potential, and pregnancy loss are well recognised. Pre-implantation genetic screening has shown an association of cleavage delay and reduced embryo morphology with embryo aneuploidy. The purpose of this study is to identify any effect of increased levels of sperm aneuploidy on embryo development and morphological quality.

28 couples in which female age was less than 36 years and who were having Intracytoplasmic Sperm Injection (ICSI) treatment were studied. The level of sperm aneuploidy was evaluated using five-probe Fluorescent In-Situ Hybridisation (Chromosomes 13, 18, 21, X, Y) on the sperm preparation performed for ICSI by density gradient separation. 2000 sperm were evaluated in each case. Embryos were assessed at 24 hours after ICSI for early cleavage, at 42 hours for the presence of >3 blastomeres, and where available, at 62 hours for >5 cells and on day 5 for development to the blastocyst stage. The embryos were graded for morphological quality on each day of evaluation.

For cases with sperm aneuploidy <2%, 33% of the 82 embryos had cleaved to be 2-cells by 24 hours after injection compared with 14% for the 72 with 2.0–2.9% sperm aneuploidy and 7% for the 28 with 3.0–6.0% sperm aneuploidy. The incidence of embryos having >3 cells at 42 hours after injection was 78%, 54% and 43% for sperm aneuploidy levels <2%, 2.0–2.9% and 3.0–6.0% respectively. At 62 hours the incidence of embryos having >5 cells was 77% for <2% sperm aneuploidy and 37% for 2.0–2.9% sperm aneuploidy. Blastocyst development occurred in 42%, 15% and 0% of the embryos for the three sperm aneuploidy levels. The percentage of day 2 embryos showing good embryo morphology was 53% for <2% sperm aneuploidy, 15% for 2.0–2.9% sperm aneuploidy, and 21% for 3.0–6.0% sperm aneuploidy. On day 5 the percentage of embryos showing good morphology was 27% for <2% sperm aneuploidy and 15% for 2.0–2.9% sperm aneuploidy.

The study reveals slower achievement of embryo developmental milestones and decreasing embryo morphology and with increasing levels of aneuploidy in the sperm preparation is used for their insemination. Sperm aneuploidy testing may be a worthwhile investigation in couples who consistently produce embryos with substandard morphology or growth rates.

M-097 Endometriosis
New approaches to increase efficiency of IVF at endometriosis associated infertility

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The Research objective was to establish how much significant the factor of hypoaandro- genic for therapeutic potential IVF is, and in what measure this factor is influenced by additional use in cycles of a testosterone-containing preparation of AndroGel at patients with is abnormal lowered (< 1 nmol/l) level of the general testosterone.

104 patients have been included in researches with endometriosis, passing treatment in IVF with use of the standard long protocol with agonist GnRg and HCG. At all patients in the past surgical treatment of an endometriosis was carried out. Patients have been distributed in 3 separate groups which in the subsequent were compared among themselves at an estimation of indicators of efficiency of IVF: group A: 57 women with level of testosteron ≥ 1 nmol/l; group B1: 24 women with level of testosterone < 1 nmol/l testosteron-treated by “AndroGel”; group B2: 23 women with level of testosterone < 1 nmol/l which were not receiving “AndroGel”.

At an estimation of parameters cycles it is revealed that in group B1 the stimulation period has made 13.9 ± 0.5 days, in group A 12.7 ± 0.5 days and in group B2 12.8 ± 0.6 of days. In group B2 course dose FSH was 3614 ± 127 ME, in group A 3083 ± 122 ME and in group B1 21,1 % and in group B2 33,3 %. Pregnancy rates on embryo growth rates were compared among themselves at an estimation of indicators of efficiency of IVF: group A: 57 women with level of testosteron ≥ 1 nmol/l; group B1: 24 women with level of testosterone < 1 nmol/l testosteron-treated by “AndroGel”; group B2: 23 women with level of testosterone < 1 nmol/l which were not receiving “AndroGel”.

It is established that low values of the general testosterone (less than 1 nmol/l) at operated in the past concerning an endometriosis of patients associate in IVF programs with deterioration of parameters cycles and decrease in efficiency IVF. Besides, appointment testosteron in the form of gel the patient with level of the general testosterone < 1 nmol/l is capable potential the effect of AndroGel, providing substantial improvement of parameters IVF cycles and approach of indicator of efficiency IVF to the level registered at patients with levels of general testosterone of ≥ 1 nmol/l.
M-099 Endometriosis
New strategy for treatment of endometriosis – combination therapy with GnRH agonist and oral contraceptives
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Objective It is well known that endometriosis is a persistent disease and easy to recur after treatment. Present study was performed to elucidate whether the continuous oral contraceptives (OC) administration was possible after three times of the preceding Gn-RH agonist.

Materials and Methods 87 cases of severe endometriosis (r-ASRM scores: 108.5 ± 30.2) were subjected. They were divided into two groups at random. Group A: 3 times of GnRH agonist (Luproyprolin) administration preceding OC (mono-phasic OC), 46 cases, and Group B: Only the OC administered without preceding GnRH agonist, 41 cases. The treatment was continued for a year after the OC administration, and the effect of the treatment was elucidated by the laparoscopic findings (r-ASRM score), the pelvic examination (Beecham’s score), and the grade of the low abdominal pain using the VAS (visual analogue scale) score. The changes of plasma gonadotropin and estradiol levels were also compared.

Results The uterine bleeding could not be found in 10 cases of the group A (21.7%) for a year, while there was no case in the group B. The period from the OC administration to the beginning of uterine bleeding between these 2 groups (eliminated former 10 cases in the group A in this comparing) was 79.5 ± 18.6 vs 53.3 ± 19.7 days, and there was a significant extension in the group A (p < 0.025). The r-ASRM scores were improved both in the group A and B (A: before: 102.7 ± 21.4, after: 47.8 ± 22.5; B: 110.2 ± 33.3, 62.9 ± 30.0), and the r-ASRM scores in the group A showed a significant decrease compared to those in the group B (p < 0.05). The lower abdominal pain and the Beecham’s score was A: 9.6 ± 2.4 → 1.5 ± 0.9; B: 9.5 ± 1.9 → 2.6 ± 0.5; A: 3.5 ± 0.9 → 1.2 ± 0.4; B: 3.7 ± 0.8 → 1.5 ± 0.6, respectively, and the better improvements tended to be seen in the group A. There was no significant difference in plasma various hormone levels during breakthrough uterine bleeding periods (FSH: 1.6 ± 0.2 vs 1.5 ± 1.1, LH: 1.7 ± 0.5 vs 2.0 ± 0.8 mIU/ml, E2: 26.2 ± 7.2 vs 28.6 ± 9.7 pg/ml).

Conclusion The new administration method, preceded GnRH agonist before the OC’s continuous administration, is effective for treatment of endometriosis through the mechanism of decreasing the incidences of the breakthrough uterine bleeding.

M-100 Endometriosis
Adenomyosis and infertility
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Introduction Adenomyosis is a benign disease of the uterus characterized by ectopic endometrial glands and stroma within the myometrium. It is associated with myometrial hypertrophy and may be either diffuse or focal. Possible association with infertility, early pregnancy loss and preterm labor.

Diagnosis Ultrasound diagnosis, hysteroscopy, menstrual biopsy, CA 125 and MRI.

Diagnosis can only be proven by the pathologists.

Adenomyosis and infertility
• Strong association between adenomyosis and lifelong infertility in the baboon [Barrier et al., 2005]
• Association between pelvic endometriosis and adenomyosis 54% [de Souza et al., 1995] to 97–90%
• Increased preterm labor
• Uterine hypermotility: alteration of sperm transport
• Altered oxidative stress
• Increased microvessel density
• Altered gene pattern expression
• Fewer follicles and corpora lutea
• MII oocytes with scattered chromosomes
• Cytoplasmic fragmentation
• Formation of pseudopronuclei
• Spontaneous oocyte activation
• Reduced fertilization and abnormal pronuclei
• Delayed-arrested embryo cleavage
• No microtubules in blastocysts.

Adenomyosis and assisted reproduction
• When to offer IVF?
• Does it affect IVF outcome?
• Is medical therapy preIVF useful?
• Should ICSI always be used?
• If surgery is needed, which technique?

Management Medical treatment, surgical approaches, uterine artery embolisation and thermal therapy.

Conclusions In women with adenomyosis the receptivity of the eutopic endometrial to embryo implantation appears normal.

Adenomyosis might impair the mechanism of directed sperm transport. Adenomyosis might compromise the intrafollicular development of oocytes and thus represents a causal factor of subfertility. Adenomyosis is strongly associated with endometriosis and uterine fibromas, thus being frequently diagnosed in infertile patients.

Implantation in ART is not affected as ascended by oocyte donation programs.

How these alterations affect the window of implantation in natural cycles is not known.

Alterations in the gene expression pattern of the endometrium of women with adenomyosis have been described.

The infertility in women with adenomyosis is best treated by hormonal stimulation and IVF, not by insemination.

Tuesday, September 14

T-002 Endometriosis
Laparoscopic management of moderate to severe endometriosis as the most effective approach to achieve pregnancy in infertile endometriosis patients
Badei M
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Endometriosis impedes or prevents pregnancy achievement and response to controlled ovarian stimulation/IVF. The outcome of assisted reproductive technology in infertile patients with endometriosis is much worse than that in unexplained infertility. In this article, we present a case of infertility associated with moderate to severe endometriosis who achieved live-birth full-term pregnancy termination after laparoscopic unipolar electroexision of endometriosis followed by just one cycle of controlled ovarian stimulation with timed intercourse, emphasizing the beneficial role of laparoscopic excision of endometriotic lesions immediately before attempting controlled ovarian stimulation with or without IVF.

T-001 Endometriosis
Laparoscopic management of moderate to severe endometriosis as the most effective approach to achieve pregnancy in infertile endometriosis patients
Badei M
Ministry of Health & Medical Education, Mehr Hospital, Gynecologic Surgery & Infertility, Tehran, Islamic Republic of Iran

Endometriosis impedes or prevents pregnancy achievement and response to controlled ovarian stimulation/IVF. The outcome of assisted reproductive technology in infertile patients with endometriosis is much worse than that in unexplained infertility. In this article, we present a case of infertility associated with moderate to severe endometriosis who achieved live-birth full-term pregnancy termination after laparoscopic unipolar electroexision of endometriosis followed by just one cycle of controlled ovarian stimulation with timed intercourse, emphasizing the beneficial role of laparoscopic excision of endometriotic lesions immediately before attempting controlled ovarian stimulation with or without IVF.
Yeast betaglycan inhibit development of experimental endometriosis and induces apoptosis of endometrial stroma

Ali F.M., Abdel Shafy A.
Ain Shams University, Cairo, Egypt

Objective: Yeast betaglycan as an antitu- morogenic, antiproliferative, antiangiogenic, anti-inflammatory, cholesterol lowering effect, this study evaluate yeast betaglycan on mouse model of endometriosis and on culture of human endometrial cells.

Design: In vivo and in vitro experimenta- tion.

Materials and Methods: Human proliferative phase endometrial biopsy were established as organ culture or used for human endometrial stromal cells to established endometriosis in ovariectomized mouse, endometrial tissues were maintained in Oestradiol for 24 hours and subsequently injected intraperitoneal, Mice randomly as- signed to receive yeast betaglycan and saline (study group n = 20, control group n = 20).

The animal were then sacrificed and endome- trial implants were measured and stained (tunnel for endometriosis).

Results: Mice treated with betaglycan de- tect a significant lower number of endome-trial implants rather then control p < 0.01, tunnel staining for implants in the study group is more than control group p < 0.01.

Conclusion: Yeast betaglycan decrease endometriosis as well as induce apoptosis in human endometrial stromal cells, this will open a novel treatment for endometriosis.

T-003 Endometriosis
Yeast betaglycan inhibit development of experimental endometriosis and induces apoptosis of endometrial stroma

Ali F.M., Abdel Shafy A.
Ain Shams University, Cairo, Egypt

Objective: Yeast betaglycan as an antitu- morogenic, antiproliferative, antiangiogenic, anti-inflammatory, cholesterol lowering effect, this study evaluate yeast betaglycan on mouse model of endometriosis and on culture of human endometrial cells.

Design: In vivo and in vitro experimenta- tion.

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Conclusion: Yeast betaglycan decrease endometriosis as well as induce apoptosis in human endometrial stromal cells, this will open a novel treatment for endometriosis.

T-004 Endometriosis
Different treatment options of ovarian endometriosis

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Objectives: Endometriosis is a condition when endometrial-like cells are present in ar- eas outside the uterus and are influenced by hormonal changes and respond similarly as normal endometrial cells.

Aim: To evaluate efficacy of different treat- ment patterns of ovarian endometriosis.

Materials and Methods: A prospective study was conducted at the Private Unit of Obstetrics and Gynecology “Minerva”, in 22 women with ovarian endometriomas, of 26 women with diagnosed endometriosis of differ- ent localization during last 5 years. Treat- ment options were: operative laparoscopic evacuation and destruction of endometriotic ovarian lesions, treatment with a (GnRH agonist, treatment with Gestron, a non- estrogenic contraceptive, and treatment of endometriomas with transvaginal puncture, aspiration and local application of Methotrex- ate.

Results: Of all patients, 18 were infertile. In 9 of them we performed transvaginal punc- ture of endometriotic cyst with local applica- tion of Methotrexate, 7 were conservatively treated with Gestronine, and 6 had laparosco- pic surgery with consecutive treatment with GnRH agonists. In 3 of them we detected re- current endometriotic lesions on the other ovary afterwards. There was no recurrence of endometriomas after puncture, or after treat- ment with Gestronine. 7 of them conceived spontaneously, while others underwent pro- grams of assisted reproduction.

Discussion and Conclusion: Endometrio- sis is typically seen during the reproductive years, in about 5% to 10% of women, and in about 30% is a cause of infertility. They are usu- ally located on the ovaries, Fallopian tubes, or on the surrounding of genital organs (pelvic peritoneum), but can be some times dispersed throughout all abdomen. Endo- metriosis can lead to anatomical distortions and adhesions, which are reason for the symptoms of this illness. Patients who rup- ture an endometriotic cyst may experience an acute abdomen. The goal of medication treatment is to block the production of estro- gen (Danocrine and GnRH agonists, progeste- rone and lately Gestronine). Lately minim- ally invasive surgery procedures are most popular because they can be performed with- out hospitalisation, such as cyst puncture with Methotrexate application, as they ap- proved their efficacy.
Conclusions So, nociceptive pain need hormonal or standard treatment and neuropathic pain need treatment too like gabapenta- nin, duloxetine or presacral neuroectomy and better the combination of both. The evidence to date, in all but one study, strongly indicates that treating endometriosis does not improve fertility. Unless significant struct- ural abnormalities are present, most studies suggest that the best approach to fertility is to ignore the endometriosis.

T-006 Endometriosis
Laparoscopic ovarian cystectomy of endometriomas: Surgeons’ experiences may affect ovarian reserve and live-born rate in infertile patients with in vitro fertili- zation-intracytoplasmic sperm injec- tion

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Study Objective To assess whether the laparoscopists’ experience can affect ovarian reserve and pregnancy outcome in vitro fer- tilization-intracytoplasmic sperm injection (IVF-ICSI) patients who previously under- went laparoscopic conservative treatment for ovarian endometriomas.

Design Retrospective cohort report.

Setting Tertiary care university hospital.

Patients A 149 IVF-ICSI cycles with infertile patients who previously underwent laparoscopic conservative treatment for ova- rian endometriomas were enrolled. There were 76 cycles in inexperienced surgeon and 73 cycles in experienced surgeon.

Intervention Controlled ovarian hyper- stimulation and IVF-ICSI.

Measurements and Main Results Primary outcome measures were antral follicle count, number of oocytes, fertilization rate, and good-quality embryos for transfer. Sec- ondary outcome measures were the impact of surgeon-dependent variables on clinical preg- nancy rate, implantation rate, and live-born rate. The number of antral follicle count (7.5 ± 3.8 vs. 9.6 ± 6.6, respectively; p = 0.011), and live-born rate per cycle (9.3% vs. 32.9%, re- spectively; p < 0.001) were significantly lower in the inexperienced group compared with the experienced group. However, the mean number of oocytes, metaphase II oo- cytess, fertilization rate, the mean number of embryos transferred, the rate of good quality embryos transferred, implantation rate and clinical pregnancy were similar between both groups.

Conclusions The experience of laparosco- pist may affect ovarian reserve and live-born rate after treating ovarian endometrioma in infertile women with IVF-ICSI.

T-007 Endometriosis
A rare association of endometri- oma and dermoid on a single- ovary patient. Case report and review of literature

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We present a case of ovarian endometriomas associated with dermoid cyst. This is a case of 30 years patient, Para 1, ad- mitted in the hospital for ovarian mass on a single ovary. She had previously been oper- ated for ectopic pregnancy associated with dermoid cyst of the right ovary, which re- quired right adnexitomy. The ultrasound scan described at present increased left ovary of 6 cm, with 2 different cystic structures: one homogenous of 40/30 mm, and one of 30/44 mm heterogenic mostly anechoic with an intracystic hyperchoicographic image. The CA 125 was increased – 45 IU/l, and therefore the surgical exploration was de- cided. It showed both an endometrioma and a dermoid cyst, the internal echo being a tooth fragment. A double cystectomy was per- formed, and ovarian tissue was spared, in re- lation with patient’s age and wish for future pregnancies.

In conclusion, we think this case presents a peculiar ovarian pathology association, in a patient in which CA 125 and ultrasound as- pect were misleading regarding the nature of cysts. There is only one such case described in the literature of bilateral dermoid cyst with endometrioma and, the difficulties of diagnosis require its presentation.

T-008 Endometriosis
Anti-A-enolase autoantibody as a novel serum marker for endo- metriosis

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Objective To establish novel serum marker for endometriosis, serum autoanti- bodies (autoAbs) were investigated using proteomic approach.

Design Retrospective study.

Setting Departments of Molecular Pathol- ogy and Obstetrics Gynecology in Ehime University and University hospital.

Interventions autoAbs in sera of endometriotic patients and healthy controls were investigated using mesothelial cell line, two- dimensional gel electrophoresis and Western blotting. Proteins in reacted spots were iden- tified using MALDI TOF-MS with Mascot analysis. ELISAs were established using re- combinant proteins and autoAb-titers were estimated in sera of endometriotic patients, disease controls and healthy subjects.

Main Outcome Measures Identification of serum autoAb useful for diagnosis of endometriosis.

Results Several autoAbs were identified. ELISAs were established and serum autoAb titers were estimated. Anti-alpha-enolase (Eno1)-autoAb levels in endometriotic pa- tients were elevated significantly compared to both healthy and disease controls. Sensi- tivity and specificity of serum anti-Eno1- autoAb was nearly comparable to serum CA125. Anti-Eno1-autoAb did not elevate in ovarian cancer patients. When anti-Eno1- autoAb and CA125 assays were combined, diagnostic sensitivity and accuracy im- proved.

Conclusions Serum anti-Eno1-autoAb can be a new serum endometriotic marker and it is useful as a supplement assay for CA125. This study validates further clinical evalua- tion of this novel marker.

T-009 Ethics and moral issues
Is high pregnancy rate the only indi- cator of success of IVF treatment? Infertility treatment analysis, Rep- roductive Unit, Department of Obstetrics and Gynecology, Uni- versity Medical Center Ljubljana, Slovenia, 2006–2008, and related issues of biomedical ethics

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Introduction Since 1986, Slovenia has been providing full access to treatment in in- fertile couples, regardless of the treatment program, including full reimbursement of costs by the state and allowing re-inclusion in the program after each delivery.

As in 2008 the European Parliament called on member states to ensure the right of couples to universal access to infertility treatment, and as ESHRE has concluded that reimbursement policies may have a signifi- cant impact on the accessibility, we think that the Slovenian infertility treatment system could be considered as an example of good clinical and ethical practice, in which justice and equality are highly valued.

We present the results of pregnancy rates (PR) in different groups of patients and inter- pret them through the basic principles of bio- ethics.

Aim 1. Specifically define the positive right to IVF treatment as the important human right connected with fundamental human rights (right to life, right to found a family).

2. To promote the value of every single life conceived, and
3. To encourage ESHRE to adopt guidelines on reporting PR by not allowing to exclude couples with poor prognosis for which IVF treatment is proven to be beneficial.

Each exclusion (beyond futility cases) should in this respect be seen as a failure to provide the right to IVF treatment, the right to life and the right to found a family.

Materials and Methods We analyzed 3247 IVF cycles performed during 2006–2008. Women were divided according to age (< 38 vs ≥ 38 years) and to their ovarian stimulation response (≤ 3 retrieved oocytes vs > 3 retrieved oocytes).

Results In low responders aged ≥ 38 years, the PR was 9.0% and the delivery rate (DR) was 5.9% (both above futility). Comparison of PR between normally and low responding women by two age groups (< 38 normal vs < 38 low; PR 29.4% vs 14.8%) (≥ 38 normal vs ≥ 38 low; PR 18.1% vs 9.0%) showed significant differences (p < 0.01).

Conclusion A fully accessible system of infertility treatment can deliver notable success also in women in which the prognosis of treatment is very poor (e.g. age > 38 years, low ovarian response), showing its additional positive effects (medical, ethical and social). Offering successful and beneficial assistance to individuals who tend to be excluded from reimbursement for treatment (either on the grounds of low cost/efficaciousness or ratio or aiming to achieve/report best overall PR) should therefore be highly valued.

T-010 Ethics and moral issues Ethical issues in ART and mass media
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Objective The aim of the present study is to demonstrate the impact of different mass media sources like films, TV series, advertising and opinion polls, on ethical aspects of Assisted Reproductive Techniques.

Materials and Methods An advertising TV spot that shows an insemination procedure of an infertile couple and its relation to the articles of the Universal Declaration on Bioethics and Human Rights, adopted by acclamation on October 19th, 2005 by the 33rd session of the General Conference of UNESCO, was analyzed. The scene takes place in a Clinic of Assisted Reproduction. A doctor talks with a woman in a corridor opposite a locked door of an office. The door opens and a boy appears. His face looks fatigued and satisfied at the same time; he hands over a vessel with sperm to the doctor. The woman hugs him and it is obvious, that this is a couple who turned to a clinic for an artificial insemination. The doctor and the woman head for another office where the process of insemination will be presumably completed. But when they are left alone inside the room, the woman adopts a provocative pose and starts to undo her blouse. The doctor does the same while he throws the vessel with sperm away. The scene finishes with the husband waiting outside with a look of hope in his face. A text that says “Life sucks” appears and then another one says “Have a pint”. This is a publicity of an Irish beer.

Results The analysis of the advertising TV spot exposed controversies regarding ethical contents of the following articles: art. 3 (Human Dignity and Human Rights), art. 4 (Benefit and Harm), art. 6 (Confidentiality), art. 8 (Respect for Human Vulnerability and Personal Integrity), art. 9 (Privacy and Confidentiality), art. 10 (Non-Discrimination and Non-Stigmatization), art. 11 (Social Responsibility and Health), and art. 16 (Protecting Future Generations). Besides the articles violated on the advertising TV spot analyzed, it was also found that it might affect negatively infertile patients’ sensitivity, promote wrong ideas about ART procedures, give a wrong image of doctors and have an adverse effect on couples that need ART.

Conclusions The impact of mass media is extremely strong and most people are not aware of this condition unless they are involved in the same situation shown. Professionals who work with mass media should pay more attention to this kind of situations since these sources could provoke an important distortion of reality with negative ethical consequences.

Currently in Poland very hot debate has been carried out which may lead to the legislation which in practice can seriously restrict access to infertility treatment i.e. to in vitro fertilization on religious grounds.

T-012 Ethics and moral issues Validation of Iranian version of the prenatal attachment inventory
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Background Feto-maternal attachment or prenatal attachment can be defined as “the unique, affectionate relationship that develops between a woman and her fetus”. This feeling may predict the future relation between mother and child and also can be affected by many influencing factors including ART and donation. Prenatal attachment inventory (PNI) was introduced by Mother in 1993. This study was designed to generate the Farsi version of PNI and validate it for Iran.

Methods First translation, back translation and re-translation were made by English language expert followed by checking and editing the translated text literally followed by review by multidisciplinary experts upon Iranian culture. Then a process of face validity was performed in a sample of different ordinary women pregnant and not pregnant. Then content validity was performed and the inventory was tested on 237 pregnant women gathered by stratified sampling from clinics, obstetricians’ offices, governmental and private hospitals and health centers in Tehran. All of the cases were pregnant for the first time and their gestational age were 27–34 weeks. Complicated pregnancies, IVF and history of chronic diseases were excluded.

Results Cronbach’s alpha in our study was 0.79 means acceptable internal consistency. Questions were devided into two groups of “mind” and “act” according to their definitions. Cronbach’s alpha for mind and act groups were 0.736 and 0.731 respectively. We considered question “I love my child” as an indicator of psychological relation and attachment between mother and unburned child. Positive correlation Coefficient can be an indicator of content validity of the questionnaire (p = 0.0001; r = 0.28). In comparison between 2 groups of questions (mind and act), the correlation coefficient for mind group (p = 0.0001; r = 0.414) and act group (p = 0.003; r = 0.193) both were statistically significant indicating content validity for each group separately. There was no significant difference between wanted and unwanted pregnancies, different ages, education or mother’s and father’s job. We just could find a positive correlation between the time from marriage to pregnancy with attainment in the mind questions group (p = 0.003) but not in the act group (p = 0.91).

Conclusion Farsi version of prenatal attachment inventory is standard tool for in-
Investigating feto-maternal attachment in Iran and can be confidently used for such researches.

**T-013 Ethics and moral issues**

**Preservation of fertility in healthy women – An ethical assessment of the implications of “egg freezing”**

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**Introduction and Objectives**

Egg freezing by means of vitrification is a technique in which (un)fertilized egg cells are frozen, stored and thawed, offering women in their fertile phase of life and, more importantly, beyond it in their postmenopausal phase of life, completely new options of shaping their lives. Although egg freezing has started as a therapeutic method, e.g. helping women with cancer to get pregnant with their own oocytes after successful cancer therapy, it could become a technology of enhancement: what should the physician answer, when the patient formerly suffering cancer now asks for her cryopreserved oocytes at age 58? This question becomes more and more urgent considering that freezing eggs is already being marketed by fertility clinics as a certificate of guarantee for healthy women to postpone their family planning. The method could enable postmenopausal pregnancy to become a widespread practice – which leads to new challenges for society (e.g. changes in generational structures). What is needed, is an ethical reflection on the impact of egg freezing, not only for every single woman, but also for society and the understanding of physician’s role in society.

**Methods**

Arguments for and against the preservation of fertility in healthy women in the current ethical debate will be analyzed against the background of the four ethical principles developed by Beauchamp and Childress (respect for autonomy, nonmaleficence, beneficence, justice).

**Results**

First, the treatment-enhancement distinction, which is often used in ethical discourses about the legitimacy of reproductive medicine, will not help to answer the question of legitimacy of egg freezing. Second, we need a much broader ethical perspective: just focusing on the four principles does not help to get the problem in focus. We have to consider that freezing eggs is already being marketed by fertility clinics as a certificate of guarantee for healthy women to postpone their family planning. The method could enable postmenopausal pregnancy to become a widespread practice – which leads to new challenges for society (e.g. changes in generational structures). What is needed, is an ethical reflection on the impact of egg freezing, not only for every single woman, but also for society and the understanding of physician’s role in society.

**Conclusions**

Egg freezing is not just a method to help women with cancer to get pregnant in later stages of their reproductive phase of life. Its social impact is much more tremendous: the preservation of fertility – especially in healthy women – does have the potential to change our society’s structure and the way we live our lives. Therefore, it is not enough just to use Beauchamp and Childress’ 4 principles in order to evaluate the impact of egg freezing. We need a broader ethical view, which is based on anthropological-philosophical reflections.

**T-014 Ethics and moral issues**

**Embryo adoption, a therapeutics options for public hospital in developing countries**

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Almost 10–15% of couples are infertile, and of this group near to 25% of them require ART, specifically IVF. But this therapeutics options is very expensive (US 3000), this economics aspect transform this therapeutics option in no valid or very expensive for Public Health System in Developing Countries like Chile. In Chile we have just 2 public center for IVF, and they do 340 cycles per year, just 1 cycle per couples, and just for women less than 37 years old. So we have around of 37.385 couples per year without options for will be mother (in base to our population). In Chile, the median salary is US $ 1100 /per month, and less than 1% of our population win more than US $ 3000 per month, and more than 80% win less than US $ 540 per month. With this problem, we have a lot of couples than need IVF and a Public Health System without money for offer them, the Embryo adoption is a real option for Public Health System in developing countries. Transfer of Frozen Embryos in natural cycle is quit cheap, around of US $ 300 or less, the problem so, is where we can obtain the frozen embryos? The answer is easy from developed countries. The Donor of Frozen Embryos, for developing countries like Chile, is a good (cheap and easy) therapeutics option for poor couples of Public Health System, that in other circumstances no have other choose.

**T-015 Ethics and moral issues**

**An investigation of women’s experiences of an IVF egg sharing scheme for somatic cell nuclear transfer research**

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With the worldwide rapid developments in human embryonic stem cell (hESC) research there is a steady need for human eggs and embryos to conduct this scientific research. Whilst hESC research promises much for future medical therapies, questions arise over the social and ethical aspects of this work.

Much debate has been given to the moral status of the human embryo, but other practices such as somatic cell nuclear transfer (SCNT) enlarge the debate and shift it to encompass the potentially additional complexity of acquiring eggs from IVF treatment. First, additional concerns over SNCT occur, e.g. fears of the slippery slope from therapeutic to reproductive cloning; whether the practice of SNCT challenges definitions of the embryo; whether SCNT still has scientific validity. Second, from the perspective of the IVF couples, providing eggs might increase the complexity for the couples involved, e.g. do they understand what they are consenting to, do they understand the goals of SNCT research; what views of eggs do they hold?

Both sets of questions are added to by the introduction of a scheme in the UK whereby women undergoing IVF are allowed to volunteer to “share” their eggs in exchange for reduced IVF fees. Thus, additional questions have to be considered: does a reduction of IVF fees constitute an inducement to donate, might that lead to an exploitation of the poor and vulnerable; does egg sharing under such circumstances actually amount to egg selling?

In our presentation, we will outline initial findings from a qualitative interview study in this field: “An investigation of women’s experiences of an IVF egg sharing scheme for somatic cell nuclear transfer research”. This is the first empirical study of this scheme. Our study investigates the views, values, and experiences of those coming forward to provide eggs, in order to evaluate whether the potential scientific and therapeutic gains from SCNT research are achieved at social and ethical costs to egg sharers. Semi-structured interviews are used to explore these issues.

First preliminary results and procedural steps from this UK Medical Research Council funded study will be presented.

**T-016 Ethics and moral issues**

**Oocyte donation and pregnancy risk: A case report**

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Literature on oocyte donation for women with primary ovarian failure (POF) more often concerns psychological attitudes and ovarian response to different gonadotropin schedules in donors, in vitro fertilization outcome and analysis of costs rather than the physical and psychological conditions of recipients. A 42 year old female, affected by primary amenorrhea of unknown origin, came to our observation to perform a replacement therapy, after she had undergone a successful IFV by oocyte donation, performed in a foreign country, with a schedule of gonadotropins without previous administration of gonadotropin analogue, unnecessary due to the POF. She had been previously treated by estrogen and progesterone replacement therapy; the evaluation of karyotype and a laparoscopy showing right ovary agenesis and small fi-
Spermbank itself like gene banks dehumanizes human life and dignity. The artificial insemination (“give life and give hope”) sounds like “donate blood and save lives”) may give hope to desperate parents to have children. Yet there is lot of difference between saving lives and giving life. Giving life carries social responsibility, which needs to take into account the child’s rights.

T-018 Ethics and moral issues
Knowledge and attitudes of women towards multiple embryo transfer, fetal reduction and multiple pregnancy
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Background Multiple pregnancy and preterm delivery are well known complications of IVF/ICSI treatment. Fetal reduction is also performed in cases of high order multiple pregnancy. There is increased impetus on transferring fewer embryos, preferably only one in younger women.

Materials and Methods 186 women who conceived following IVF/ICSI treatment participated in a questionnaire study regarding their knowledge and attitudes towards multiple embryo transfer, fetal reduction and multiple pregnancy.

Results A majority of women said that they were aware of the complications of multiple pregnancy (90%) and preterm delivery (85%). Nevertheless none of them opted for a single embryo transfer. A positive pregnancy test was more important to most women than the outcome of that pregnancy (74%). Fetal reduction did not pose any moral concerns to most women (67%). Anxiety about the safety of the remaining twins persisted throughout pregnancy (73%). Having twin babies did not affect the quality of life of most women (74%).

Conclusion Indian women were similar with their western counterparts in desiring multiple embryo transfer in order to maximize their chance of getting a positive pregnancy result. The negative impact of twin or higher order pregnancy appears to disregard by the women prior to getting pregnant. The confidence of the treating physician to offer single embryo transfer also appears to affect the patients’ choices.

T-019 Ethics and moral issues
Attitudes of HIV positive people toward parenthood and their desire to have child: A focus group qualitative study in Iran
Omami Samani R., Mohraz M.
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Introduction Efficacy of antiretroviral medication has improved life expectancy of the HIV positive people leading to increase their desire to have family and children. In Iran, almost all the centers refuse HIV positive people. At the end of 2006, the cumulative number of the reported cases of HIV/AIDS was 13,702 and it is expected to be higher in the society. This study was designed to determine HIV positive people attitude toward family and child.

Methods The study was a focus group qualitative study. 30 HIV positive people were gathered in a round table meeting and detailed discussion made about the subject. All the session was filmed after getting permission and ensuring the participants not to broadcast the film and use it just for research. For gathering different ideas, thanks to AIDS research center, we gathered as different people as we could. For example we invited discordant couples, discordant couples, single people, drug abuse HIV infected, recently married after diagnosis and previously have a child. Detailed discussion took around 3 hours and continued during the dinner.

Results Almost all of the cases believe that reproduction is a right for them if the child can be born healthy, but no one accepted transmission of the virus to the child. Desire to have family and children, was obvious among them, although many of them could not believe that it may happen. Many of the cases pointed to the child as a source of joy and happiness, hope and self steam for the family. Many of the participants believed that HIV is similar to other chronic diseases and even better when HIV is positive but there is no AIDS. Almost all of the cases admitted stigmatization of HIV in our country and isolation of HIV positives from the society, but they believe that everything is changing. Considering welfare of the child, most of the cases believed that HIV positives can be good parents and raise their children even better than many healthy people. A few participants pointed to the possible children embarrassment in their community and among the relatives because of their HIV positive parents. All the cases accepted the donation, surrogacy and adoption choices to reach a healthy child.

Conclusion The desire of Iranian HIV positive people to have family and children is significant. It is necessary to offer them safe assisted reproductive services to prevent their isolation from society, transmission of the virus to the child and losing their hope and self steam.
T-020 Ethics and moral issues

Egg donation and egg sharing, which one is more ethical and religiously acceptable?

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Iran is the only Islamic country in which donation programs are practiced. Egg sharing which was defined in 1992 as considering infertile patient as egg donors to other patients in exchange of subside in their treatment expenses. We designed this study to compare egg sharing to ordinary egg donation from ethical, legal and Islamic view. A complete survey of ethical issues and debates about egg donation and sharing was done. Religious issues were followed by a clergy from Islamic texts and current decrees (fatwas). Social issues and legal problems were evaluated by expert opinion and law review. Also a complete review of the cases sent to the courts was done. Egg donation and egg sharing are both accepted religiously in Iran by the Shiite clergy, but no donation is accepted by Sunni Muslims. Upon to Islam regardless of Shiite and Sunni, look and touch to the female genitalia is forbidden unless there is an absolute necessity which for normal donor, the necessity is under question. Short term and long term complications of fertility drugs and procedures are more acceptable in sharing. Problems like repetitive donations, price and money exchange, advertisement and brokers all can be minimized in egg sharing program. There are some disadvantages about the egg sharing like possible psychological burden for egg sharer after treatment failure brought by the thought that maybe the “recipient succeeded”, or she always can haggle with herself that “this procedure reduced my chance and maybe the treatment does not work”. They can be prevented by a good consultant. Another disadvantage is possible minor and may be undetectable health problems in the infertile women that could be transferred to the offspring or make failure in the treatment. This can be prevented by careful consideration of the egg sharers. We think that the only ethical way for obtaining the eggs for research can be egg sharing. Finally it seems that egg sharing is an ethical way for Catholics to use the extra oocytes in an altruistic way and prevent destroying the extra embryos, and also for those countries in which cryopreservation of the embryo is forbidden. Conclusion We can conclude that egg sharing is ethically, religiously and legally preferable to egg donation. Also, it can help the researchers to obtain their need for eggs more ethically.

T-021 Ethics and moral issues

Patient attitudes toward the fate of their frozen embryos after the end of cryopreservation time

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Background This study was designed to investigate the couples’ decisions regarding the fate of their frozen embryos after the preservation period and the underlying reasons for their decisions.

Methods A list of the infertile couples who had frozen embryos in the Nitrogen Tanks of Royan Institute was made. All of them have passed the five-year period of cryopreservation according to the institute legislation. A semi structured questionnaire was designed to get their ideas freely in response to our questions. Data were gathered through telephone calls by an expert staff well trained for preventing any bias in the interviews.

Results From all our cases 78% chose donation to other infertile couples, 12% to research, 5% discard and remaining 5% could not choose any of the proposed fates for their embryo even after enough time for thinking and at the end they left the decision to us. Most of the people had altruistic incentives for donating the embryos and on the contrary for not donating, the main concern was the destiny of the resulting child. Among the possible influencing factors like age, education, duration of infertility, previous failure(s) in treatment, abortion history, job and current live child, the only factor was to have a child (p = 0.009) that was associated with higher choice of donation to the other infertile. Among these cases 70% believed that the recipient is the mother, 11% step mother, 1% not related and remaining 15% could not specify any relation between the child and the recipient, but regarding relation between the child and the donor 41% believed in no relation followed by 19% don’t know, 19% mother, 16% genetic relation and 4% step mother. Also among them, 54% were interested to know the child, 43% know the recipients and 61% had considered some characteristics among the recipients to donate them their embryos. Also, in the group of cases who decided to donate for research, the type of preferred research or education usage of their embryos was decided as follows: 88% chose embryology, 91% stem cells and 81% educational usage. All the people who decided to discard the embryos were worrying about the destiny of the resulting child and the consequences of the researches.

Conclusion Unlike other parts of the world, infertile people in Iran mostly like to donate their surplus embryos to other couple rather than donating to the research or discarding them.

T-022 Ethics and moral issues

Embryo reduction: Islamic view

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Background Multiple pregnancies are identified as complications of ART treatment, in some conditions it is necessary to reduce the number of fetuses. In this study we investigated religious aspects of it from Islamic view.

Methods Our method was to review all the Islamic texts both Shiite and Sunni and find out proofs about this procedure. Also we have gathered decrees (Fatwas) of clergy leaders about it.

Results As the embryo-reduction to some extends is similar to abortion, all the ideas about the abortion is presented:
1. An embryo is not considered a human unless 40–50 days of age by some Sunni clergies and 120 days of age by the Shitites.
2. An embryo in the womb is respected and abortion is forbidden unless some special circumstances.
3. In Iran, there is a law permitting the abortion when it is medically necessary, which indication is confirmed by Iran’s forensic medicine court.
4. After ensoulment (enter of the soul from God to the fetus), the fetus is considered a human and abortion is considered killing a human and has the full penalty. At this age, even Iran’s therapeutic abortion law does not support the necessary abortion, but termination of the pregnancy in any age is accepted if the mother’s life is in danger. As the embryo reduction is a necessity made by previously performed operation, if the procedure considered a sin, people who produced the necessity to commit a crime are responsible too. So, there must be absolute necessity for transferring more than one embryo or excessive use of induction medications in IUI, which can lead to multiple pregnancies. We could not find absolute necessity of transferring frequent embryos because there are plenty of researches supporting single embryo transfer. Also, there can be some ways to reduce multiple gestations made by IUI that must be followed. Upon decess of our clergy leaders, although it is a sin and must be prevented, but as there is a necessity for saving the other children, it is acceptable. Upon Sunni clergies despite of ensoulment time of 40–50 days, because of necessity and saving the lives, it is accepted.

Conclusion Embryo reduction can be accepted by Islam both Shiite and Sunni because of necessity and saving the lives. Multiple pregnancies must be prevented even if the success rate drops and the clinical team is responsible for the religious consequences of this procedure if they do not follow the guidelines for preventing multiple pregnancies.

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Conclusion Embryo reduction can be accepted by Islam both Shiite and Sunni because of necessity and saving the lives. Multiple pregnancies must be prevented even if the success rate drops and the clinical team is responsible for the religious consequences of this procedure if they do not follow the guidelines for preventing multiple pregnancies.
The Donor Sibling Registry (DSR) is a non-profit, web-based, worldwide organisation dedicated to educating, connecting and supporting those affected by gamete donation, including donors, recipients and offspring. At 25,000 members, the DSR has connected 7,000 genetic first degree relatives; hundreds of donors enjoy contact with offspring and thousands of half-siblings interact together.

However, the DSR does not just generate genetically-related joy – it also shines light on serious genetic concerns about gamete donation. Frequently, the DSR counsels recipients whose children have inherited undisclosed genetic disorders, or who have discovered their donor was dishonest regarding health, or that the sperm bank did not notify them about reported illness or amended the medical profile.

The number and severity of these health matters is discomfiting. Since donors can father many offspring (one DSR donor has more than 120 known offspring) donors can transmit disease to scores of children- around the world. Ranking only second to seeking contact with genetic relatives, DSR members cite interest in sharing or warning about health issues. The DSR is the only facility whereby donors, recipients and offspring can unrestrictedly and immediately share medical information. Thousands use it for this purpose.

Currently, many sperm banks either refuse to update donor/offspring medical information or, even if they accept updates, refuse to share the information, or make the process of reporting so complex or expensive that donors and recipients simply cannot comply or afford it. Amazingly, in this era of genomic sequencing, some US sperm banks do not afford it. Amazingly, in this era of genomic sequencing, some US sperm banks do not afford it. Amazingly, in this era of genomic sequencing, some US sperm banks do not afford it.

Materials and Methods A semi-structured questionnaire was sent to the parents of DI children, after obtaining preliminary confirmation that they would be willing to answer. The entire procedure was exactly the same as that used in the previous survey, however, parents who included in the previous survey were excluded from the present study.

Results Out of the 154 questionnaires sent to consecutive parents of DI children, 73 responded and 42 indicated their willingness to cooperate. 27 husbands (64%) and 30 wives (71%) returned their answers during a 2-month period. Although most of the parents had no intention to disclose this fact (husbands 73%; wives 79%), 3 husbands (11%) and 2 wives (7%) intended to disclose about the DI in future. Furthermore, less husbands (63% vs 77%, comparing with previous report) and wives (59% vs 75%) thought that DI origin should be kept secret to children. The main reason for this lack of desire for disclosure was that both believed that the person who supports the family and loves the child as his own should be considered as the father (927%, 12/30). The most important person of sources which influenced the parents’ thinking about disclosure and the children’s knowing rights were healthcare personnel, especially their doctors (51% for husbands, 37% for wives), and the information from internet.

Conclusion A larger number of parents seemed to be more deeply concerned about the circumstances surrounding disclosure than in the previous study. Although Japanese parents are still not as open to disclose their DI origin to the children as in other countries, the attitude seems to be changing.

The case for comprehensive medical testing of gamete donors

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Donor Sibling Registry, Nederland, United States

Introduction Anonymous-based donor in-semination (DI) is the only authorized gamete donation in Japan. Although no deleterious effect in familial relationships or the mental development of children has been recognized, the rights of DI children to know their genetic origin have become a topic of discussion and debate worldwide. Although our previous study, performed in 2002, indicated that almost none of the couples were willing to disclose to their children about DI, a movement has been started by children conceived through DI and concerned doctors and nurses to increase the awareness about disclosure and the rights of DI children. In this context, we investigated the attitude towards disclosure among the parents of DI children in 2008, and compared the results with those described in our previous report.

Methods The 2 sources for the data compared in this study (referring to 2005 and 2006) were FIVCAT.NET (an official compulsory Assisted Reproduction Registry within the Health Ministry of the Regional Government of Catalonia, to which all authorized clinics, both public and private, performing assisted reproduction in the region are obliged to report) and the register of the Spanish Fertility Society (SEF), to which data are provided on a voluntary basis. The SEF register data were divided into two groups: 1) data from clinics in Catalonia (SEF-CAT); 2) data from the rest of Spain, excluding Catalonia (SEF-wCAT). The techniques compared were patients’ own versus donor egg cycles.

Results For cycles with patients’ own eggs, the voluntary ART register reflected 77.2% of those on the official one, but the corresponding figure was only 34.4% with respect to donated eggs. The variables analyzed in own-egg cycles (insemination technique used, patients’ age, number of embryos transferred, pregnancy rates, multiple pregnancies and deliveries) were similar in the three groups studied. However, we observed significant differences in donor egg cycles with regard to the insemination technique used, pregnancy rates and multiple pregnancies between the voluntary and the official register.

Conclusions From the voluntary ART register for own-egg cycles are valid, but those for donor egg cycles are not. Further study is necessary to determine the reasons for this difference.
T-026 Fertilization
The role and importance of contamination control on the success of in vitro fertilization

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The importance of contamination control and cleanliness on the success of in vitro fertilization process has not deeply been understood in Germany. But more and more the specific clinics understand the rules and necessities. The result is a 50% higher success rate.

Not only the particulate cleanliness but also very important is the volatile organic content (VOC) in the air, meaning the molecular contamination with carbon vapors. In the end the fertilization clinic operates even cleaner than most pharmaceutical productions with all the paper work and good manufacturing requirements. An example will be given and some recommendations will be given.

T-027 Fertilization
The effect of stress on infertility

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This is an article on the effect of stress on infertility.

According to specialists in obstetrician, enormous stress can be one of the causes of infertility psychological changes caused by stress, the feeling of anxiety as well as depression can result in infertility by influencing hormones and reproduction. The failure of pregnancy results in increasing stress as well as missing the power of fertility more.

Stress disturbs sleeping cycles. Insomnia changes several hormones related to reproduction and fertility. Psychological stress is a result, not a cause of the fertility of people. Also stress in women disturbs the hormonal relationships among the brain, pituitary gland and ovary by interfering with the process of the ovule ripeness.

The negative effects of the stress in women is remarkably more than men.

Changing this statement “I will never have a baby” into “I will do everything to be pregnant and have a baby” can reduce stress and anxiety and cause changes in the heartbeat and the hormone cortisol and eventually the power of fertility increase.

Researches have shown that there is a meaningful relationship between the hormone adrenaline and depression. Psychological treatments (behavioural, cognitive and psychotherapy) at the moment of treatment and diagnosis and especially before IVF and pregnancy test result in making the pregnancy test positive; in addition, using the mental treatments increases the chance of pregnancy even after 6 month continuation.

Working stress as well as competition for working success make some changes in hormones and estrogen available in blood of women is replaced by androgen containing the hormone masculine testosterone. The increase of the amount of androgen is accompanied by virilism and decrease in the power of fertility.

As a result, with regard to the crucial importance of the role of stress in infertility it is beneficial to carry out research on this subject and the ways to cope with stress.

T-028 Fertilization
Post swim-up sperm count as a predictor for fertilization-failure in patients undergoing IVF with normozoospermia

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Objective The risk of total fertilization failure (TFF) with conventional insemination during IVF treatment is reported to be as high as 20% but the exact cause is not clear in most cases. This study was performed to find out whether there is any correlation between the post-swim-up sperm count and TFF after IVF.

Study Design A retrospective cohort analysis.

Methods In this study, 116 patients with normozoospermic semen samples who underwent IVF between Jan and Sept 2009 were analyzed. Based on indication of IVF, 116 patients were classified into 2 groups of unexplained infertility (n = 48) and all tubal pathologies (n = 68). All patients with less than 3 oocytes were excluded from the study. Semen preparation techniques for all normozoospermic samples employed density gradient technique, followed by 2 washes and a final swim-up for exactly 10 minutes. After quantifying the post-swim-up yield, 80,000–150,000 sperm were added per 4–5 oocytes for overnight fertilization. 2 further groups A and B were made based on post-swim-up motile sperm concentration. Group A included 18 patients with “Poor swim-up” with motile sperm concentration less than 4 M/ml. Group B included 98 patients with “Good swim-up” with sperm concentrations > 4 M/ml. Mean Pre-wash motile sperm concentrations in both groups were 37 ± 6.5 M/ml and 44 ± 7.2 M/ml respectively and statistically similar.

Results Overall, 12 out of the 116 patients (10.5%) resulted in TFF. There was no difference in the rate of TFF in patients with unexplained infertility and tubal factor infertility being 10.41% and 10.29% respectively. However, in the “Poor swim-up” group, out of 18 patients, 6 had TFF (33%) whereas in the Good swim-up group, 6 patients out of 96 (6.3%) resulted in TFF.

Conclusion IVF with poor post swim-up sperm concentration resulted in 33% total fertilization failure, which was statistically higher (p < 0.01) than 6% rate of fertilization failure in group of patients with good post swim-up sperm concentrations. Post swim-up motile sperm concentration may be an effective tool to predict TFF.

T-029 Fertilization
Analysis of the anti-zona pellucida (ZP) antibodies in follicular fluids (FF) from patients treated by in vitro fertilization (IVF)

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Objectives Some idiopathic POF patients had anti-ZP antibodies in their sera, detected by the microdot assay using a human ZP [Fertil Steril 2007; 88: 925–32]. In this study, we investigated the incidence of anti-ZP antibodies in FF and also the effect of the antibodies on sperm-ZP binding by the hemizona assay. Moreover, we examined the relationship between the presence of the antibodies and the incidence of 2 pronuclei (PN) formation in IVF cycles.

Design and Methods 19 sera and 123 FF were obtained from patients treated by IVF. For the microdot assay, ZP were prepared by unfertilized human oocytes that failed to fertilize in vitro or that were not used because of being immature. After hundreds of oocytes were stored, the ZP were separated from their cytoplasm mechanically and they were then placed in PBS at 75°C for 30 minutes. The microdot assay was an immunomodulating method to detect the anti-ZP antibodies. The color development of each dot was evaluated by computer-assisted image analysis by NIH.

NIH image number (NIN) was calculated by subtracting the density of the background stain from that of the dot stain.

Results 1. 8 of 19 (42.1%) sera and 4 of 123 (3.3%) FF reacted with soluble ZP.
2. 2 of 8 (25%) patients with antibodies in the sera possessed the same antibodies in FF. However, none of 11 patients without antibodies possessed the antibodies in FF.
3. In case 1, 2 of 4 (50%) FF reacted with soluble ZP. In case 2, 2 of 13 (15.4%) reacted with it. These FF were collected from the large follicles.
4. Incidences of antibodies in FF for positive and negative in the sera were 7.1% (4/56) and 0% (0/67). There was a significant difference between the 2 groups (p = 0.04).
5. In patients with higher NIN (≥ 3500), all of 4 patients with positive FF and 3 of 20 (15%) patients with negative FF inhibited sperm-ZP tight binding (HZI < 50%).
6. Incidences of 2 PN for positive and negative antibodies in FF were 100% (4/4) and 78.6% (55/70). There was no significant difference between the 2 groups (p = 0.576).
Conclusion The positive rate of anti-ZP antibodies in FF was lower than that in the sera. None of patients without the antibodies in the sera possessed the antibodies in FF. The incidences of 2 PN for positive in FF were 100%. The present findings might contribute to the diversity of the inhibitory effects on fertilization by antibodies. Further studies are required to investigate the antibodies by SDS-PAGE analysis.

T-030 Fertilization Optimisation of TESE-ICSI by injection of laser-selected immotile sperm in Zona score selected oocytes

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Objectives Intracytoplasmic sperm injection (ICSI) relies on selection of vital sperm. In azoospermic men sperm are retrieved by testicular sperm extraction (TESE). In many cases only immotile sperm are collected. No distinction can be made between dead and immotile vital sperm by light microscopy. However, a laser shot directed to the tip of the flagellum allows distinction of viability since the flagellum forms a coil at the site of impact. These laser selected sperm are used for injection of oocytes. Quality of oocytes can be assessed by light microscopy using standardized criteria regarding the appearance of cytoplasm, the Zona and the abundance of vacuoles. Recently, the application of polarisation microscopy (PolarAIDETM Octax) was applied to rank the quality of oocytes according to their Zona score which correlates with implantation and pregnancy rates. Here we analyze whether the introduction of sperm and egg selection criteria improves ART outcome under the German law (embryo selection is forbidden; only 2–3 polar stages can be cultivated).

Methods We compared 38 TESE-ICSI cycles (n = 34 with number of sperm ≥ number oocytes, n = 4 with less sperm than oocytes) in year 2008 (group 1, G1) with 32 TESE-ICSI cycles in year 2009–2010 (n = 29 with number of sperm ≥ number oocytes, n = 3 with less sperm than oocytes, group 2, G2). In 2009 laser selection of immotile sperm and Zona scoring of oocytes were introduced.

Results In G1 we collected and injected motile sperm in 24 out of 38 cycles; in 8 cycles motile and immotile and in 5 cycles only immotile sperm could be retrieved from TESE samples. In G2 we obtained motile sperm in 17 out of 31 cycles, in 10 cycles motile and immotile and in 2 cycles only immotile sperm were found. In G1 the fertilisation rate (FR) with motile sperm was 54.1% (146/270 oocytes). In G2, ICSI using motile sperm and application of the Zona score increased the FR up to 57.7% (120/208 oocytes). The injection of immotile sperm into oocytes in G1 resulted in a FR of 17.7% (11/62 oocytes) while the introduction of the laser selection in 2009 increased the rate up to 29.8% (14/47 oocytes) in G2. The overall biochemical pregnancy rate (bcPR) was in G1 12.9% and in G2 it increased up to 21.4%.

Conclusion FR and bcPR in TESE-ICSI can be enhanced by laser selection of immotile sperm and by Zona scoring of oocytes. When motile sperm are available oocyte selection by Zona score alone had already a positive effect.

T-031 Fertilization Administration of intramuscular progesterone, vaginal progesterone and 17a-hydroxyprogesterone caproate (17-HPC) in patients undergoing in vitro fertilization-embryo transfer: A Prospective randomized study

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Purpose The objective of this study was to compare the progesterone in oil, intravaginal progesterone and 17a-hydroxyprogesterone caproate in pregnancy rate in women undergoing in vitro fertilization embryo transfer cycles.

Material and Methods A prospective randomized study was performed in Royan Institute between March 2005 and March 2007. The Inclusion criteria were the use for GnRH down-regulation and age < 40 years. The Exclusion criteria were ART cycle cancellation, contraindication of progesterone and the cycles with ART treatment failure. 154 patients were assigned to receive one of the treatments.

Results No differences between groups were found in total characteristics. The endometrial thickness on embryo transfer day was similar (0.93 ± 1.41, 0.9 ± 1.55 mm, p = 0.92). No statistical significant was found for biochemical pregnancy (37.3%, 36.5%, 28.3%; p-value = 0.56) and observation of gestational sac (35.8%, 34.6%, 26.4%; p = 0.55) between 3 groups.

Conclusions Our study manifested the effect of three types of progesterone were similar on pregnancy rate.

T-032 Fertilization Regular menstrual cycles and spontaneous pregnancy after hematopoietic stem cell transplantation (HSCT) and high-dose chemotherapy: Case report

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Background Premature ovarian failure and subsequent infertility are among the most common complications of hematopoietic stem cell transplantation (HSCT). Risk for ovarian damage depends on patient’s age, type and cumulative dose of drugs administered, with busulphan and cyclophospham being the most hazardous for ovarian reserve, and concomitant irradiation. Until now, only 2 cases of spontaneous pregnancies in women with history of HSCT have been reported.

Case Report A 21-year-old female referred to our center for general examination. Her past medical history was significant for acute myeloblastic leukemia diagnosed in 1998. In 2001, when the patient was 14 yo, HSCT was performed and high-dose chemotherapy, including busulphan 16 mg/kg, cyclophospham 120 mg/kg and daunoz 200 mg/kg, in combination with preventive cranial irradiation was subsequently started. At the moment chemotherapy was begun menarche had yet not occurred. After HSCT hypergonadotropic hypogonadism developed. The patient received HRT within 1 year after completion of chemotherapy. After HRT was discontinued, regular menstrual cycles appeared. FSH and LH concentrations upon referral were 5.4 and 4.4 IU/L respectively, AMH level reached 0.6 ng/ml, E2 level – 102 pmol/l. US scan remonstrated dominant follicle 18 mm in the ovary and patient conceived spontaneously in that cycle. At gestational age 9–10wks missed miscarriage (fetal karyotype 69, XXX) was diagnosed. Regular menses restored after manual vacuum aspiration and the second spontaneous pregnancy occurred 4 months later. At the moment of abstract submission gestational age was 18wks and pregnancy course was uncomplicated. General examination showed no pathology and laboratory tests had normal results. Biochemical screening in the 1st and the 2nd trimester revealed no abnormalities. Fetal development corresponded well with gestational age.

Discussion Current data shows that 50% of women with history of HSCT at the age of 11 and older never have menarche. Only 2 cases of spontaneous pregnancies in women with history of HSCT have been reported globally. We present a unique case of a patient in whom HSCT was performed at the age of 14 and still regular menstrual cycles appeared and 2 spontaneous pregnancies occurred.
T-036 Fertility preservation
Prevalence of preventable infertility causes in infertile woman from an academic tertiary infertility care
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Objective To evaluate the possible preventable infertility causes and their prevalence in infertile women from an academic tertiary infertility care.

Methods Retrospective analysis of medical records of 98 patients treated at the outpatient infertility clinic at the Universidade de Caxias do Sul, form January 2007 to July 2009. We evaluated infections, alcohol, smoking, weight and illicit drugs as possible causes of infertility in our infertility setting.

Results The age of woman ranged from 18 to 45 years (average 29.8 years). The reported time of infertility ranged from 5 months and 20 years. Only 17.3% patients reported condom use during life. Regarding to sexually transmissible illnesses, no patients tested positive for HIV, syphilis, hepatitis B or hepatitis C. Eight patients (8.2%) reported Human Papillomavirus infection and 11 (11.2%) Chlamydia trachomatis infection. Active Chlamydia trachomatis infection was diagnosed in 30.4% and others 30.4% presented with reagent titre suggestive of prior infection. 2 patients had a history of use of illicit drugs previous to the infertility investigation. The alcohol use was reported by 22 patients (22.4%), and tobacco by 31 patients (31.6%). Others 19 patients (19.4%) stopped smoking. 51 patients (52.0%) had a BMI outside the range considered normal by the World Health Organization (WHO). Among these, 19 patients (19.4%) were obese, 28

T-035 Fertility preservation
Bilateral teratoma management in women with fertility preservation desire
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Introduction Germinal tumors represent a 15 to 20% of the ovarian tumors. Formed by three germinal tissues that come from the embryonic layers to form it, and could be the cause of serious ovarian damage with their reproductive consequences.

History In 1659 it was described as a finding in an autopsy of a young lady. The first time that the term “dermoid” was used was in 1831.
(28%) were overweight and 7 (7.1%) was underweight.

**Conclusion** Considering that 60.8% of the patients presented with *Chlamydia trachomatis* infection, 31.6% were smokers, 19.4% obese, and that the age, smoke, infections (mainly the infection for *Chlamydia trachomatis*) and important alterations in the BMI are infertility causes that may be prevented with simple measures such as campaigns of fertility preservation, it is relevant to state that any public politics for intervention in reproductive health may avoid infertility in these women. Prevention programs with education, support and accessibility to health professionals for guiding the population regarding these infertility issues may help patients to maintain their fertility.

**T-037 Fertility preservation**

**6 lines for fertility preservation in cancer therapy**

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**Introduction** The incidence of cancer in pre and post-puerperal women is about 8%, chemo and radiotherapy may induce transient or persistent amenorrhea and premature ovarian failure. This raise the need to preserve the fertility of oncological patient to allow a reproductive life after cancer remission. Here we report our experience in the period from 2006–2009 in many lines, 1st line: Comparing the efficiency of ovarian tissue cryopreservation using a conventional slow freezing medium and a new media replacing sodium (autologous eye tears), 2nd line: Telomere elongation based on the fact that reproduction is proportional to telomere elongation. 3rd line: Uses of laparoscopy to inject substances in the ovary before and at the beginning of chemo and radiotherapy, 4th line: improving the fertility preservation by combinations of first cryopreservation of ovarian tissue followed directly by ovarian stimulation. 5th line: Use of Nano technology in animal experimentations. 6th line: Human follicles development can be achieved by Bio engineering culture system.

**Conclusion** For the first time in literature we introduce autologous eye tears replacing sodium to improve cryopreservation of human ovarian tissues, elongation to improve reproduction, laparoscopic intraovarian injection of PBMCN, Bee venom, cerebrolysin, melatonin to improve the ovarian reserve, and the combination of first cryopreservation of ovarian tissue followed directly by ovarian stimulation allowing doubling the efficacy of ovarian stimulation before chemo and radio therapy. Nanogold particles protect the ovarian follicle from radio and chemotherapy, the strange in nanogold particles is that by its use we reduce the radiation and chemotherapeutic doses and lastly Bio engineering culture system for Human follicles development.

**T-038 Fertility preservation**

**Partial trachelectomy: A new approach for fertility-preserving management of cervical ectopic pregnancy**

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**Aims** Although the application of methotrexate (MTX) and the development of interventional radiology has made uterine preservation possible for cervical ectopic pregnancy in most cases, hysterectomy is typically performed as the final measure to treat patients when conservative therapy has failed. By contrast, radical trachelectomy is often selected in recent years, for early invasive cervical cancer to maintain the fertility of the patients. The aim of this study was to develop a fertility-sparing surgical procedure for cervical ectopic pregnancy.

**Methods** The patient was a 26-year-old woman, gravida 2, para 0, who was diagnosed of cervical ectopic pregnancy with gestational age of about 8 weeks. Although 6 courses of MTX treatment made fetal heart beats arrest and serum β-human chorionic gonadotropin (β-hCG) level reduce from 187497 IU/ml to 414 IU/ml, vascularity increased around the deformed gestational sac and massive hemorrhage frequently became to occur. Curettage, which was performed with angiographic balloon-occlusion of bilateral uterine arteries, caused uncontrollable hemorrhage. Then, the surgery “partial trachelectomy” including:

1. Interruption of blood supply by ligating the bilateral descending branches of the uterine artery.
2. Circumcision of the vaginal fornix, partial resection of cervical wall with the ectopic product of conception.
3. Reconstruction of the cervix and anastomosis between the vagina and cervix was attempted to spare potential fertility and the efficacy of this procedure was evaluated by observing the postoperative course.

**Results** Her serum β-hCG value dropped to below detectable levels shortly after the surgery and menstruation returned after one month. Magnetic resonance imaging at 6 months post-surgery showed a normal corpus and small cervix.

**Conclusions** This potential fertility-preserving surgical technique should be applied when it is considered difficult to treat using ordinary conservative therapy, including MTX administration, or when hysterectomy is indicated to save the life of a mother who still hopes to preserve her uterus.

**T-039 Fertility preservation**

**Laparoscopic surgery of adnexal mass in obstetrics**

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**Introduction** Adnexal cysts situated on the 2 place, among all tumors of women reproductive system and often leads to abnormal pregnancy. Usual surgical technique for these patients is laparotomy.

**Materials and Methods** 377 pregnant patients were divided on 4 groups: 1 group include 241 patients who was undergoing surgical laparoscopy in 16–18 weeks of gestation; 2 group include 41 patients with laparotomy in 16–34 weeks; 58 patients from group 3 were delivered abdominal by caesarian section and tumor removal; laparoscopic procedures at 37 patients from 4 group were done on Day 4–5 after vaginal delivery. Gestation period at 16–18 weeks is optimal for surgery, because placental formation is finished already and small uterine size makes laparoscopy possible.

Laparoscopy in pregnant patients have several characteristic: "open" laparoscopic method by 2 cm minilaparotomy without Veress needle, low level of pneumoperitoneum, untypical trocar port places, only short-term relaxation is possible. 2–3 days preoperative period tocolytic therapy were done.

**Results** 268 patients from group 1 and 2 were successfully vaginal delivered, 5 patients are still pregnant, 9 patients were delivered by caesarian section. In group 3 adnexal mass became an indication for abdominal delivery in 26 (45%) cases.

**Conclusion** Early diagnostics of ovarian cysts during pregnancy, detection optimal period for surgery, gently surgical technique, rational obstetrical tactic of pregnancy treating may decrease pregnancy abnormalities and rate of caesarian section.

**T-040 Fertility preservation**

**Management of fertility-preservation in breast-cancer-patients < 40 years in a large breast cancer center**

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**Introduction** The increase of breast cancer in young women < 40 years and the increasing age of women at the time of the birth of their first child underlines the importance to implement counselling for fertility-preserving strategies in the management of breast cancer care. The aim of this study was to present the fertility preserving procedures performed after routine counselling for primary breast cancer patients in a large breast

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cancer center (approx. 600 primary cases per year).

Materials and Methods Between November 2006 and November 2009 52 patients < 40 years with histologically confirmed breast cancer were counselled for the fertility-preserving possibilities before breast surgery and chemotherapy in the fertility-center of the university women’s hospital.

The therapeutic options for fertility preserving methods were based on the tumor characteristics, the planned treatment, the time until start of the oncologic procedure and the recommendations of the network FertiPROTEKT.

Results In 36 months 52 primary breast cancer patients years were seen in the fertility center. The medium age was 32.1 years. 38 patients (= 73.1%) had hormone receptor-positive tumors.

The majority of the patients (n = 22) decided for ovarian tissue cryopreservation as fertility preserving method. The laparoscopic approach was normally combined with the oncological-surgical procedure. GnRH-protection was performed in 13 patients. In 10 patients an ovarian stimulation protocol was initiated to cryopreserve fertilized or unfertilized oocytes. A combination of different fertility-preserving methods was performed in 11 patients.

Conclusions In contrast to other studies, the majority of young primary breast cancer patients were hormone receptor positive. Because of this special tumor entity the possibilities to perform fertility-preserving methods is very limited especially in those patients, who have to undergo neoadjuvant chemotherapy, since it is unknown, whether high estradiol-levels caused by controlled ovarian stimulation will influence the disease. The only therapeutic option for fertility preserving methods in this patient group is the ovarian tissue cryopreservation.

Main source of funding Women’s university hospital

T-041 Fertility preservation Successful term-delivery following medically managed 9 weeks live caesarean scar pregnancy

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Objectives Implantation of a pregnancy within uterine musculature is considered to be the rarest form of ectopic pregnancy and constitutes a life threatening condition. Here we present such a rare case and the skillful medical management with a live birth in the subsequent pregnancy.

Methods A case report – 34 year old G5P1A13 presented with 45 days amenorrhoea with persistent pregnancy inspite of attempted medical abortion. She had one live child by LSCS & underwent hysterotomy later for failed MTP elsewhere. USG on 10.08.07 at Rao Hospital showed live fetus of 9 weeks CRL. 2.1 cm located in the anterior myometrium at the level of int. os. Endometrial Cavity & cervix delineated separately. BHCG was 58,000. Under GA & TVS guidance KCI injected into fetal heart & FH stopped. Inj. Methotrexate 50 mg IM given. On 14.08 BHCG was 17,000. Inj. Methotrexate repeated. BHCG on 21.08.07 was 6,210. Patient was discharged after an uneventful stay of 10 days. On 21.08.07 BHCG was 1260, on 30.10.07 BHCG was 9 units. On 15/11 patient menstruated & USG showed no gestational mass. Patient conceived 6 months later. Antenatal period was uneventful & she delivered by LSCS a healthy baby of 2.5 kg at 36 wks on 8/4/09.

Discussion Pregnancy in uterine scar is a rare form of ectopic and can be managed medically or surgically with an aim to conserve fertility in a hemodynamically stable patient. & C is not safe due to the possibility of severe intraoperative hemorrhage which may be life threatening & requiring emergency laparotomy. Wedge resection of scar gestation with uterine artery embolisation has been reported. Our patient with uterine scar pregnancy was managed with KCI into fetal heart and 2 doses of IM MTX, had an excellent outcome as evidenced by drop in BHCG from 58000 to 6210 in 10 days & BHCG came down to 9 units in 90 days after intervention. Gestational mass disappeared around 110 days of intervention. She conceived within 6 months after resolution of scar pregnancy. Serial ultrasound was the single most useful tool for monitoring this uterine gestation which was uneventful.

Conclusion From our case study it proves that a skillful medical management of a live uterine scar pregnancy can result in conservation of uterus and fertility.

T-042 Fertility preservation Vitrification and xenografting of human ovarian tissue

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Background Ovarian tissue cryopreservation and transplantation are becoming increasingly important issues for preserving fertility for young women with cancer. We reported that viable follicles were observed in the human ovarian tissues vitrified by DAP protocol and xenografted into the ovarian bursa of Non-obese diabetic severe combined immunodeficient (NOD-SCID) mice. More recently, Kagawa et al. reported successful vitrification of bovine and human ovarian tissue by their Cryotissue protocol [Reprod Biomed Online 2009; 18: 568–77].

Design and Methods Human ovarian tissue was obtained from 64 consenting female-to-male transsexuals (30.7 ± 5.6 yo; mean ± SD). This study obtained approval from Saitama Medical University Ethical Committee. Ovarian cortex tissues were cut into slices with the size of 1x10x10 mm, which is suitable for transplantation in clinical situation. In DAP protocol, the tissue slices were first incubated in PBI medium containing DMSO at 4C for 5 min, then incubated in DAP 213 solution containing DMSO, acetamide, and propylene glycol at 4C for 5 min, and plunged into liquid nitrogen. In Cryotissue protocol, the tissue slices were incubated in an equilibration solution containing ethylene glycol (EG) and dimethyl sulfoxide (DMSO) at 26C for 25 min, then incubated in vitrification solution containing EG, DMSO and sucrose at 26C for 15 min, and plunged into liquid nitrogen. The frozen/thawed human ovarian tissues were xenografted into the ovarian bursa or the dorsal subcutaneum of SCID mice, and resected two weeks after for the calculation of residual follicle density.

Results 84 ± 20 follicles/mm3 (mean ± SE) in their early stage, i.e. primordial, primary, and preantral, were observed in the ovarian tissue obtained from the patients less than 40 y.o. (n = 6) before cryopreservation. In the frozen/thawed tissues by Cryotissue protocol, 67 ± 7 early-stage follicles/mm3 were observed. 2 weeks after xenografting, 10 ± 5 and 4 ± 2 early-stage follicles/mm3 were observed in the bursa and subcutaneum, respectively. No viable follicles were observed, however, in the xenografted tissues originated from the large slices cryopreserved by DAP protocol.

Conclusion Human ovarian tissue slices with ready-for-clinical size vitrified by Cryotissue protocol may have approximately 1000 viable follicles in each slice after transplantation. Overcoming the initial ischaemic damage that depletes a significant fraction of the oocyte pool will be one of our next challenges.

T-043 Fertility preservation Patient oriented approach to fertility preservation

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Introduction Fertility preservation in young cancer patients has become a matter of great concern worldwide over the last years. In Germany, the FertiPROTEKT network offers up to date advice about treatment options.

Objective To analyse the data from the first year of fertility preservation treatments at the
hospital of the Technical University in Munich within the framework of FeniPROTEKT.

Materials and Methods Retrospective observational study of 39 patients scheduled for cancer treatment that were advised at our center from October 2008 to March 2010.

Results Mean patient’s age was 31 years. 77% had not yet started any oncological therapy. The most common diagnoses were: hormone receptor positive breast cancer (18%), Hodgkin’s lymphoma (18%), acute myeloid leukemia (15%), osteosarcoma (10%), non-Hodgkin’s lymphoma (10%), and hormone receptor negative breast cancer (7%).

Fertility preservation treatment could be applied in 77% of cases (n = 30). GnRH agonists were used in 90% (n = 27), alone (n = 16) or in combination with other therapies (n = 11). Of the patients who received GnRH agonists alone, 50% had already started chemotherapy at the time of consultation. Cryopreservation of ovarian tissue was done in 33% (n = 10), whereas controlled ovarian stimulation was performed in 23% (n = 7). The protocol used was with recombinant FSH and GnRH antagonists, and it was started independent of cycle day. The mean number of gonadotropins used was 2800 IU, with a subsequent mean number of 15 oocytes. In cases where vitrification of unfertilised oocytes was done (n = 3), the mean number of oocytes was 10.3. When a fertilisation was desired (n = 4), the mean of cryopreserved pronuclei was 9 per patient. A combination of therapies was done in 36.6% of cases (n = 11); in cases which combined all three procedures (n = 2), the ovarian stimulation was initiated 2 days after laparoscopic extraction of ovarian tissue without further complications.

Conclusions Ovarian stimulation two days after laparoscopic extraction of ovarian tissue seems to be a safe option. The stimulation protocol must be adapted to the time at which the consultation takes place. Despite the lack of scientific evidence for fertility preservation with GnRH agonists, they frequently represent the only therapeutic option due to delayed consultation after chemotherapy onset.

T-045 Fertility preservation Safety procedures for preserving and restoring fertility by ovarian cortical strip autograft in cases of non-malignant diseases, and cautiousness in cases of malignant diseases: One center’s experience

Introduction Cryopreservation of ovarian tissue with subsequent autograft allows fertility to be preserved at high risk of premature ovarian failure resulting from treatments for cancer or non-malignant diseases.

Materials and Methods In our center, oophorectomy is performed after a reflection time of at least 3 days. Ovarian cortical strips are cryopreserved according to a slow cooling protocol and medullary tissue is reserved for histology. The quality of the cortex is assessed before and after freezing, by histology and trypan blue staining of isolated follicles. Additional checks before graft concern: microbiology, long-term viability and minimal residual disease (MRD) detection. A two-step orthotopic autograft is performed. Ovarian function recovery and graft survival are evaluated by hormonal levels, ultrasonography and RMI.

Results Malignant diseases represented 90% of the indications for ovarian cryopreservation. 30% of the patients or their parents refused the oophorectomy and a final medical contraindication was found in 15% of cases. After trypan blue staining, 77% of follicles were alive after thawing (versus 90% before freezing). Conventional histology disclosed ovarian metastases in only one patient (Ewing’s sarcoma). A cortical tissue dissociation method should allow us to obtain a cellular suspension, in which abnormal cells could be detected by flow cytometry in hematological diseases. One autograft was performed in a patient treated by allogeneic bone marrow transplantation (BMT) for a non-malignant disease. The re-establishment of ovarian function occurred 4 months after the graft. The patient conceived spontaneously and delivered a healthy child. Nine months after the birth, ovarian tissue had a “normal appearance” as shown by ultrasound and RMI. FSH/LH values were normal, but AMH remained low.

Conclusions Autotransplantation of cryopreserved ovarian cortex is a safe method of re-establishing fertility in cases of non-ma-
T-047 Fertility preservation

Autograft mature mouse spermatogonial cells into azoospерmia mouse model

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Introduction Spermatogenesis is a complex process involving in generation of a large of cells that eventually differentiate into sperm. The basis of this process is the spermatogonial stem cells (SSCs). Despite their importance in infertility treatments and studies, SSCs are infrequent in adult mouse testis and their proliferation is slow. Improved culture techniques, cryopreservation, and transplantation are useful tools for a better understanding of male germ cell biology and the regulatory factors of male fertility. In the present study, we examined the autograft of fresh and frozen-thawed mature mouse spermatogonial cells into azoospermia mouse model generated by gamma irradiation.

Methods Sertoli and spermatogonial cells were isolated from adult mice testes using two step enzymatic digestion and lectin immobilization. The identity of the isolated cells was confirmed by analysis of alkaline phosphatase activity, immunocytochemistry against oct-4, and vimentin and also transplantation of these cells to mouse model.

For autologus transplantation, right testis from adult 6-8 weeks old NMRI mice was removed to obtain cell suspension. Then spermatogonial cells of the resulted colonies from right testis were transplanted into the seminiferous tubules of the other testis of the same mouse that were irradiated with 14Gy at 10 weeks of age, via rete testis. The SSCs were divided into 4 groups: fresh cells (control 1), fresh cells co cultured with Sertoli cell (control 2), frozen-thawed SSCs (experimental 1) and frozen-thawed SSCs co cultured with Sertoli cells (experimental 2). The statistical significance between mean values was determined using statistical tests.

Results Our results indicated that 8 weeks after autologus transplantation, transplanted spermatogonial cells underwent proliferation and sperm production in the recipient testes. There was a significant increase in the epididimis sperm counts following colony cells transplantation of SSCs co-cultured with Sertoli cells.

Conclusion Co-culture system with Sertoli cells can increase in vitro colony formation of adult fresh and frozen-thawed spermatogonial cells. Autologous transplantation was resulted proliferation and sperm reproduc in the recipient testes.

T-048 Imaging

Imagination and adjustment disorder in infertile couples

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This research was a descriptive study carried out to assess depression and social exchanges in infertile couples. In this study, most of the 32 infertile couples suffered from depression and anxiety, when compared with 27 fertile couples. Among infertile women, 30% had depression and anxiety vs 5% of fertile women. Among men, all of them had social phobia in their workplace and were ashamed of that, whereas none of the fertile men did. From these couples 5 had been fertile after a long period of time. Before fertility, all of them believed that all their problems arise from their childlessness, and the child will resolve everything. But all of fertile and infertile couples believed that the child disturbs the balance of their daily activity. Three of infertile couples have established a special balance to bear their infertility. Infertility disturbs self-esteem, body image and sexual identity of each couple and they are obliged to establish a new balance in order to bear the emotional and social outcomes of this disability. For stress reduction the nurse must provide referral to other sources of assistance, educate and help them in decision making. The emotional problems surrounding infertility illustrate extremely well the need for a greater emphasis on family-centered care. Sometimes, exams and treatment can solve these problems. Both of partners will require regular psychological help, in addition, medical information about the reasons for their infertility and several treatments for their problem, show them that pregnancy is not goal, but preparing a healthy home for the child either physically or psychologically is the most important goal in human’s life.

T-049 Imaging

Atomic force microscopy (AFM) of heat-treated human sperm

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Aims Atomic force microscopy (AFM) employs a sharp probe for profiling surfaces with unique resolution. AFM provides high-resolution imaging of surface structures at scales ranging from a tenths nanometers to tens of micrometers. Heat stress is considered to be one of the factors of the DNA fragmentation and sperm apoptosis. The aim of the study was to use AFM for imaging the surface pathology of the sperm exposed to heat treatment.

Methods Fresh samples of semen from sperm donors were obtained. Spermatozoa
were selected by using the conventional swim-up procedure. The first sample was taken immediately after finishing swim-up at the temperature 37.0°C, the second after heating 30 minutes at 40.0°C and the third after 30 minutes at 50.0°C. Atomic Force Microscope NTgera VITA (NT-MDT) was used to characterize spermatozoa morphology within a nanometer scale. Scanning head type SFC050NLT designed for scanning by probe (maximal operation area 100x100x10 µm) equipped with a holder for operation in a liquid was used for all experiments. Glass slide with immobilized spermatozoa was fixed to the bottom of plastic Petri dish and the internal volume was filled with sterile isotonic solution of sodium chloride. Samples imaged in contact mode at ambient temperature were scanned by using silicon nitride probe with 0.01 N/m elastic constant at 0.25 Hz scanning speed. Surface topography and spatial distribution of electrical potential gradient on the sample surface were measured and recorded simultaneously, thus providing more complex understanding to the surface morphology. The defects were detected and recorded.

Results
The obtained images clearly show both normal sperm head and defects of the surface of spermatozoa exposed to heating. Even the ultrastructure at the top of the flagellum and the region of the acrosome cap are clearly distinguishable. Moreover, elements of internal structure can be observed whenever the plasmatic membrane is missing. The best images of both normal and damaged spermatozoa were selected for presentation.

Conclusion
The collected AFM images clearly highlight many details of normal spermatozoa and spermatozoa damaged by heating. This technique could be an important tool in the research of heat and oxidative stress and understanding its effect on male infertility.

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T-050 Imaging
Bio effect of ultrasound on fetus
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Primary objectives of this article are bio effects, biomedical therapy, modelling techniques that utilized vibration and acoustic. Ultrasound has had a profound influence on the practice of medicine, especially in obstetrics. It has been almost half a century since the first ultrasonic devices were developed to provide an image of the fetus. The early studies with these devices showed a potential to provide high resolution information about the fetus, information that, if obtained by other techniques, could pose significant risks. Ultrasound did not appear to be associated with any known hazards. Thus, diagnostic ultrasound also gained wide clinical acceptance because it is convenient to use, comfortable for the patient, and not very expensive. Any biological affect of ultrasound that is accompanied by temperature increments less than 1°C above normal physiological level is called a mechanical effect. However, one should keep in mind that the term mechanical effect also include process that are not a mechanical nature but arise secondarily to mechanical interaction between ultrasound and tissue, such as chemical reactions initiated by free oxygen species generated during cavitations and sonoluminescence. Pulsed ultrasound can produce damage to biological tissue through non thermal mechanism. The acoustic output used to induce these adverse bioeffect is considerably greater then the output of the diagnostic devices when gas bodies are not present. However, the low intensity pulsed ultrasound is used clinically to accelerate the tissue repair process and induce healing of nonunion of bones. Low intensity pulsed ultrasound also has been shown to enhance repair of soft tissue damage and accelerate the nerve regeneration. Although such exposures to low intensity do not appear to cause damage to exposed tissue, they do raise questions about the acoustic threshold that might induce potentially adverse developmental effects in foetus. Recently biological effect on ultrasound has been gradually clarified at molecular level and they are expected useful for medical treatment. The physical and chemical effect cause by sonocation are shown to stimulate cells to change gene. This suggest possibility to control expression by ultrasound, presumably leading to note clinical modalities.

T-051 Imaging
Comparison of three staining methods for the morphological evaluation of human spermatozoa
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Semen analysis is a keystone in the clinical workup of the infertile male patient. The evaluation of sperm morphology is one aspect of the semen analysis that is said to give a good indication of fertility potential. Morphology remains a parameter in assisted reproduction, determining which treatment is necessary as well as predicting the outcome following IVF treatment. However, staining procedures for the evaluation of sperm morphology can be time-consuming and it would therefore be beneficial if these time-consuming techniques could be abandoned in favour of equally-efficient and quicker methods. Additionally, staining technique should have as little influence on the sperm morphology as possible, as this may ultimately affect the morphology evaluation of borderline forms. The main objective of this study was to compare the resultant standard morphological parameters (mean percentages) of neat semen after staining smears with Papanicolaou, RAPIDIFF® or D -4000® by analysing the slides sent to both WHO and Tygerberg Strict Criteria using the SCA. Additionally, we wanted to investigate the mean morphometry measurements of the different staining techniques to determine if shrinkage or swelling of the sperm occurred. The percentage of normal sperm forms were significantly higher in the RAPIDIFF® stained slides than that of the SpermBlue® stained slides when assessed according to WHO criteria. No significant differences were detected between the morphologies of the different stains when compared according to Tygerberg Strict Criteria. We noted that morphometry readings were significantly different between the groups where in general, RAPIDIFF® caused the sperm to swell, whereas the use of the Papa- et al., 2009). Combined with new technologies, these findings we suggested that clinicians exercise caution during morphological evaluation, as the outcome may be largely dependent on the staining technique used. Due to the lack of standardization regarding staining techniques between clinics we recommend that the stain used be indicated on the evaluation sheet.

T-052 Imaging
Optimizing the outcome of IVF using SonoAVC and new techniques for selection of gametes and embryos

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Three-dimensional transvaginal ultrasound (3-D) in combination with Automated Volume Count (SonoAVC™, GE Medical Systems, Kretztechnik, Austria) is a new technology that uses a 3-D dataset to provide an automatic estimation of absolute follicular volumes and mean diameters; it guarantees more accurate data than 2-D measurements. Our group recently demonstrated that 3-D SonoAVC™ can be effectively used to determine the day of hCG administration in IVF treatment based on the same criteria used with 2-D ultrasound [Muttlinger M, Aburumieh A, Zech M.H. et al., 2005]. Combined with new technologies such as IMSI (Intracytoplasmic Morphologically Selected Sperm Injection), which helps in better selecting good quality spermatozoa for the fertilization of oocytes, overall more embryos reach the blastocyst stage and thus improve the chances of success. Our aim is to improve the pregnancy rates in general and to take home rates. Aiming at elective single embryo transfer, new aseptic vitrification technologies guarantee high survival rates of embryos after thawing and improve cumulative pregnancy rates. Additional topics will be discussed on in vitro maturation of oocytes as well as the evaluation of embryonic development of gametes to the blastocyst stage and their...
T-053 Imaging
A threshold of endometrial thickness with a significant reduction in pregnancy rate in natural cycle frozen embryo transfer

Aims
Endometrial receptivity is one of the most important factors establishing the likelihood of success in assisted reproductive technology. The measurement of endometrial thickness (EMT) by transvaginal ultrasound is an easy to perform and reproducible method of assessing endometrial proliferation. The aims of this study are to confirm whether EMT on the day of hCG administration can be used as a predictor of successful outcome in natural cycle frozen-thawed embryo transfer (FET) without artificial endometrial preparation, and moreover, to determine the threshold of EMT indicative of unlikely pregnancies.

Methods
429 cycles from 327 patients who underwent a single FET in a natural cycle at our clinic between January 2005 and September 2009 and satisfied the study criteria were included in this analysis. Inclusion criteria consists of:

1. A rapid freezing and thawing protocol (vitrification) performed on the thawed embryo.
2. The transfer of a single good quality blastocyst-stage embryo with grade greater than stage 3, according to Gardner classification.
3. An assisted hatching technique was not performed on the thawed embryo.

Results
EMT on the day of hCG administration ranged from 5.8 mm to 20.1 mm (10.1 ± 2.1 [mean ± SD]) with the majority of cycles (89.7%) occurring between 7 mm and 12 mm. There was no correlation between EMT and patient’s age. The EMT of pregnancy group (P group) and non-pregnancy group (NP group) were compared. Furthermore, the cycles were divided into 2 groups based on an estimated cut-off threshold, and clinical outcomes were compared and analyzed between the 2 groups.

Conclusion
Ultrasound measurement of EMT is an important indicator to evaluate endometrial receptivity for an embryo in natural cycle FET and EMT is a predictor of fecundity. Moreover an EMT of less than 8 mm represents a threshold indicative of unlikely pregnancies in natural cycle FET.

T-054 Imaging
Controlled ovarian hyperstimulation and intrauterine insemination cycles in patients with unilateral tubal blockage diagnosed by hysterosalpingography (HSG)
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HSG is widely used as a first-line approach to assess uterine anatomy and tubal patency in routine infertility workup for tubal obstruction. Management of the patients with unilateral tubal blockage diagnosed by HSG is controversial subject. Although, a number of reports recommended laparoscopy and dye test to confirm or refute the diagnosis, then 1 reconstructive tubal surgery by laparoscopy, selective salpingography and tubal catheterization or hysteroscopic transcervical tubal cannulation. The other practitioners suggested ovarian hyperstimulation (COH) and IUI as the initial treatment of choice in patients with unilateral tubal occlusion diagnosed by HSG.

Objective
To evaluate the effect of unilateral tubal blockage (diagnosed by HSG) on cumulative pregnancy rates of stimulated IUI cycles.

Design
Cross-sectional analysis, between October 2006 and October 2009.

Setting
Academic reproductive endocrinology and infertility center (Mirza koochak Khan Hospital, Tehran, Iran).

Patients
2 groups of patients undergoing stimulated IUI cycles were compared. 64 uninfertile couples with unilateral tubal blockage diagnosed by HSG as the sole cause of infertility in the study group, and two hundred couples with unexplained infertility in the control group.

Intervention
The patients underwent 3 consecutive ovarian hyperstimulation (CI miphiten citrate and human Menopausal Go- nadotropin) and IUI cycles.

Main Outcome Measures
Cumulative pregnancy rates for 3 consecutive stimulated IUI cycles.

Results
Demographic data were found to be homogeneous between the study and control groups. Cumulative pregnancy rates were similar in the study group (26.6%) and the control group (28%) (p = 0.823; OR = 1.075; 95%-CI: 0.57–2.28). The cumulative pregnancy rate in subgroup with mid-distal tubal blockage (16%) was not statistically lower than subgroup with proximal tubal blockage (33%) (p = 0.15; OR = 2.625; 95%-CI: 0.745–9.25). The cumulative pregnancy rate in subgroup with mid-distal tubal blockage (16%) was lower than the patients with unexplained infertility (28%), the difference was not statistically significant (p = 0.209; OR = 2.042; 95%-CI: 0.671–6.213).

Conclusions
Controlled ovarian hyperstimulation and IUI could be recommended as the initial treatment in sub-fertile couples with unilateral tubal blockage as the sole cause of infertility.

T-055 Imaging
Three dimensional power Doppler evaluation of human endometrium after administration of oxytocin receptor antagonist (OTRa) in an IVF program
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Objectives
To compare endometrial and sub-endometrial morphological changes and vascularity based on a specific scoring system under the influence of oxytocine receptor antagonist (OTRa) during IVF cycles, by 3D power Doppler sonography. The hypothesis to be investigated was that endometrial status under OTRa administration would have a differentiated profile which might play a significant role for the implantation process.

Methods
A total of 26 women were recruited from an IVF-ET (Embryo Transfer) program. Exclusion criteria were uterine fibroids, distortion of the uterine cavity and women who had undergone a previous operation on the uterus. The inclusion criteria were women not older than 40 years who had at least one good-quality embryo, as defined by the morphological criteria on the 3rd day after oocyte retrieval. The mean age of women was 33.5 years (range 27–40 years). The predominant diagnosis was endometriosis, male factor and tubal factor. Control group (n = 13 women) was examined with 3D power Doppler 3 days after embryo transfer. The second group (n = 13 women) underwent 2 days after embryo transfer, intravenous Atosiban 7.5 mg administration and one day later a 3D power Doppler ultrasound was performed.

Results
Control group presented:
1. Echogenic endometrium in all cases
2. Endometrial thickness > 7 mm in 11 cases (84.6%)
3. Endometrial volume > 2.31 cm³ in 5 cases (38.5%)
4. Abnormal sub-endometrial hallo in 3 cases (23.1%)
5. Endometrial blood flow in 6 cases (46.2%) and f) complex vessel architecture in 2 cases (15.4%).

OTRa group had:
1. Echogenic endometrium in 1 case (7.7%), triple line endometrium in 12 cases (92.3%)
Conclusions To our knowledge, this is the first report relating specific 3D power Doppler ultrasonographic characteristics of human endometrium with the administration of OTRAs (Atosiban) on the 3rd day of embryo implantation in women with endometriosis.

**T-056 Imaging**

**64-row multidetector CT virtual hysteroscopy**

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**Aims** To evaluate the accuracy of multi-detector CT virtual hysteroscopy (VH) for the evaluation of cervical and uterine endometrial pathology in comparison with carbon dioxide hysteroscopy (H) as the gold standard method.

**Methods** 61 patients with diagnosis of infertility were prospectively studied. All patients underwent VH with a 64-row CT scanner using a power injector at 0.3 mL/sec. All patients underwent H with a power injector at 0.3 mL/sec. Patients were derived to H after VH. The studies were performed and evaluated independently and in a blinded way. MDCT images were analyzed using multplanar reformats and virtual endoscopy. The diagnostic accuracy for VH was calculated using the exact binomial method.

**Results** In the cervix, H detected 7 polyps and 2 synchiae; the prevalence of disease was 16.4%. In the uterus, H showed 1 septate uterus, 2 adenomyosis, 11 myomas, 7 synchiae and 47 polyps; the prevalence of disease was 78.7%. For cervical lesions, VH showed a sensitivity, specificity, positive and negative predictive values of 80%, 98%, 89% and 96% respectively. For uterine lesions, VH showed a sensitivity, specificity, positive and negative predictive values of 96%, 92%, 98% and 86% respectively. For all lesions, VH showed a sensitivity, specificity, positive and negative predictive values of 96%, 92%, 98% and 86% respectively. For all lesions, VH showed a sensitivity, specificity, positive and negative predictive values of 96%, 92%, 98% and 86% respectively. For all lesions, VH showed a sensitivity, specificity, positive and negative predictive values of 96%, 92%, 98% and 86% respectively.

Conclusions VH is a new non-invasive modality useful for the evaluation of the reproductive system, with excellent results in comparison to H results.

**T-057 Imaging**

**Gadolinium vs iodine CT virtual hysterosalpingography: An alternative for allergic patients?**

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**Aims** To determine the usefulness of gadolinium as a contrast agent to perform CT VHSG and to evaluate the intraluminal enhancement, image quality and patient discomfort in comparison with iodine-enhanced CT VHSG.

**Methods** 50 patients with diagnosis of infertility were studied with iodine-enhanced V-HSG (25 patients) and with gadolinium-enhanced V-HSG (25 patients) using a 64-row multidetector CT scanner. The iodine-enhanced studies were performed with a dilution of 15% in saline solution, whereas the gadolinium-enhanced studies with a dilution of 40%. Quantitative analysis was evaluated by Image quality was evaluated by the radiologist in a 0 to 10 scale. Each patient completed a questionnaire for discomfort assessment.

**Results** The mean peak attenuation during iodine-enhanced V-HSG in cervical area was 1090.15 HU (99%-CI: 1005.52–1174.78) whereas during gadolinium-enhanced V-HSG was 722 (99%-CI: 552.78–891.22) (p < 0.0001). The mean peak attenuation during iodine-enhanced V-HSG in endometrial cavity was 1127.2 HU (99%-CI: 1058.51–1195.6) whereas during gadolinium-enhanced V-HSG was 877.39 (99%-CI: 812.81–941.96) (p < 0.0001).

The mean image quality grade for iodine-enhanced V-HSG studies was 9.29 and for gadolinium-enhanced V-HSG studies was 8.83, with a difference of 0.46 (p = 0.07, 95%-CI: -0.05–0.96). No differences were found in patient discomfort between both groups.

**Conclusions** Our experience showed that gadolinium-enhanced VHSG studies had a lower intraluminal attenuation, but no difference was found in image quality and patient comfort in comparison with iodine-enhanced VSG, suggesting a good alternative for iodine allergic patients with acceptable attenuation for diagnostic purposes.

**T-058 Imaging**

**Three dimensional ultrasound in diagnosis of Müllerian anomalies presenting as the didelphys-unicollis uterus to the bicervical uterus with or without septate vagina**

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**Objective** To point out the value of three-dimensional ultrasonography (3DUS) in the diagnosis of Müllerian anomalies “without a classification” from the didelphys-unicollis uterus to the bicervical uterus with or without septate vagina and its concordance with magnetic resonance imaging (MR).

**Design** Retrospective study

**Setting** University hospital

**Materials and Methods** 28 women with congenital uterus – cervical anomalies were first examined using conventional 2-dimensional sonography or hysteroscopy. 3DUS was then performed. MR was also performed in 20 cases.

**Results and Conclusions** According 3D US, the length of vagina, cervix, could be identified. Their high accuracy and detailed elaboration of utero-cervix-vaginal anatomy guided the clinical therapy in intervention from single incision drainage, tissue flap for neovagina, peritoneal neovaginoplasty or hemihysterectomy etc.

New appropriate classification of the müllerian duct anomalies should be made for each superior and inferior uterine segment by 3DUS.

**T-059 Imaging**

**Morphometric variations and embryo cleavage kinetics associated with the insemination process**

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**Introduction** The introduction of a novel tool, the EmbryoScope, consisting in an incubator with video time lapse recording and image analysis permits the exact timing of the most important events during embryo development and accurate measurement of morphologic characteristics. The aim of this study was to determine the effect of the insemination process, classic in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), due to the zygote characteristics and early embryo division kinetics.

**Materials and Methods** In a prospective cohort study, we analyzed 149 zygotes, obtained from 32 couples undergoing oocyte donation IVF/ICSI cycles. The size of the oocytes obtained (OS), the zona pellucida
thickness (ZPT), the timing for pronuclear fusing (PFN), first and second cleavage (1CT and 2CT), and the interval between them (IB), were registered by using the EmbryoScope monitoring system (Unisense, Fertilitech, Ahrus). We defined early cleavage as those embryos which divided before 26 hours. The results were compared depending on the insemination technique (IVF vs ICSI), by using t-tests for means or chi square tests for proportions; p-values < 0.05 were considered significant.

Results The mean OS (µm) was 96.19, ZPT (µm) was 17.5, PNBD was 25.03 hours (h), 1CT at 28.19 h, the at 2CT 42.23 h, and IB at 14.02h. When we analyzed the zygote characteristics when handled for either IVF or ICSI, we found increased OS on those prepared for ICSI, 92.77 µm vs 98.71µm; p < 0.0001, in comparison with the oocytes prepared for IVF, while the ZPT remain intact, 17.5 µm vs 17.5 µm.

There were not statistical differences in both groups in PNF, 25.2 h vs 24.6 h, the 1CT 28.0 h vs 27.2 h, the 2CT 42.3 h vs 41.2 h, and the time range between the first and second cleavage division 14.2 h vs 13.8 h, for IVF and ICSI respectively. Considering the % of embryos presenting early cleavage, there were no statistical differences depending on their origin, IVF 40.9% or ICSI 46.7%.

Conclusion From our data, when time lapse tools are used to analyze zygote characteristics or embryo events timing no important differences are observed depending on the insemination technique. The wide use of image analysis and video time lapse technologies in the evaluation of the embryo processes will become a fundamental tool in order to estimate implantational potential via non invasive methods.

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T-060 Imaging Diagnosis of uterine malformations. Performance of CT virtual hysterosalpingography

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Aims CT virtual hysterosalpingography is a new non invasive technique that allows to evaluate the female reproductive system based on bidimensional, tridimensional and virtual endoscopic images. The aim of this presentation is to demonstrate and illustrate the usefulness of CT virtual hysterosalpingography (VHSG) in the identification and classification of uterine anomalies.

Methods 1000 patients (mean age: 35.4 years) with diagnosis of infertility performed a VHSG with a 64-row CT scanner (Brilliance 64, Philips Medical Systems) with slices of 0.9 mm thickness and 0.45mm reconstruction interval. 10–20 mL of contrast dilution (3 mL of iodine contrast and 17 mL of saline solution) were instilled into the uterine cavity through a fine catheter. CT images were evaluated on a workstation using multiple post-processing techniques including CT images were evaluated on a workstation using multiple post-processing techniques including multiple planar reformatted images, maximum intensity projections, volume rendering and virtual endoscopic images. CT images were evaluated for the presence of intrauterine linear, irregular filling defects consistent with the diagnosis of intrauterine adhesions (synechiae) and classified as focal or diffuse according to the area of the uterus involved.

Results Synecchiae were observed in 55 of the 790 patients studied (7%). From the 55 patients with intrauterine synechiae, 12 had history of ectopic pregnancy, 27 of abortion and 16 of surgical treatments. Focal adhesions were observed in 35/55 (63.7%) whereas diffuse adhesions were detected in 20/55 (36.3%). Multiple planar reformatted images and maximum intensity projections were useful in the evaluation of this pathology. However synechiae were better visualized using volume rendering and virtual endoscopy views. Both of them gave a precise location as well as extension of the disease.

Conclusions VHSG is a useful modality to evaluate the presence of intrauterine adhesions. Volume rendering and virtual endoscopy images gave a precise diagnosis of extension and localization of this pathology.

T-062 Implantation Treatment of repeated implantation failure by autologous eye tears

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Objectives To present for the first time in literature Autologous eye tears treatment for failed repeated implantation failure.

Setting Heliopolis research center and Heliopolis Hospital.

Patients 6 patients complaining of implantation failure.

Intervention Intrauterine injection of (10 c.c.) of autologous eye tears taken by capillary tube through embryonal Catheter at the time of ovum pick up.

Main Outcome Measure Live birth rate.

Results 4 term viable fetuses were born, two premature viable fetuses were born with no fetal or maternal complications.

Conclusion Eye tears treatment is a new modality for repeated implantation failure with no fetal and maternal complications.

T-063 Implantation Search of the early ultrasound markers of complicated course and adverse outcome of pregnancy that occurred after assisted reproduction technologies

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Due to the presence of many characteristic features of pregnancy course that occurred after assisted reproduction technologies (IVF) the problem of search of the early ultrasound markers of its complicated course and adverse outcome is still very topical. One of the perspective techniques is ultrasound examination. There has been conducted a prospective study on single embryo pregnancy course of 22 patients after IVF, who constituted the main group, and 13 patients with spontane-
ous single embryo pregnancy, who represented a control, that included dynamic ultrasound examination with measurement of the formation and condition of chorion and embryo cavity as well as the resistance index of the uterus arteries. The study revealed the changes of the above-mentioned parameters among the main group patients with subsequent spontaneous abortion. Changes in intraterus volumes of the patients after IVF with further developed placenta deficiency has not been definitely confirmed and is still in the stage of study. Based on the data obtained the following conclusions can be made:

1. During pregnancy the progressive increase of both chorion volume and embryo cavity volume takes place; the volumes of chorion and embryo cavity after IVF application significantly exceed the corresponding volumes during spontaneous pregnancy at the same terms.

2. The most significant increase of the chorion and embryo cavity volumes is observed at 14 and 16 weeks (more marked in pregnancy after IVF) that corresponds to intensive growth of an embryo and periembryo structures at the beginning of the second trimester.

3. During further regressive pregnancy more marked decrease of chorion and embryo volumes is observed compared with both progressive pregnancy after IVF and spontaneous pregnancy.

4. Blood flow in the uterine arteries in pregnancy after IVF exhibits lower level of resistance than in spontaneous pregnancy; theta has been observed some increase of blood flow resistance at 8 weeks that corresponds, on the chronologic periodization, to the peak of first wave of trophoblast invasion.

5. There has not been revealed a distinct correlation between the dynamics of chorion volume increase and its cavity as well as between changes of uterus vessels resistance with developing long-term complication of pregnancy (IVF) and its late abortion.

T-064 Implantation

hCG expression and production in endometrium of normal secretory cycle phase

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hCG and β-hCG was found in many gonadal organs. It is unclear whether the hCG expression is an epiphenomenon or whether it has a physiological significance in the tissues. In our studies we determine whether beta human chorionic gonadotrophin (hCG) (CGB) subunits and alpha hCG (CGA) subunits are expressed and the hCG dimer is produced in normal human cyclic endometrium. Endometrial specimens were collected for histological dating from women undergoing diagnostics in our division of human reproduction. RNA from normal secretory endometrium was extracted, and CGB and CGA gene expression was assessed semiquantitatively by PCR. Adequate secretory endometrial specimens were homogenized using trishaclot inhibitors. Proteins present in the supernatant were separated electrophoretically, and molecular hCG isoforms were detected by Western blot. The supernatant hCG concentrations were measured by ELISA. We characterized hCG and leukocytes in endometrial specimens by immunohistochemistry. Uterine flushing was performed to confirm endometrial hCG secretion into the uterine fluid. hCG should not be considered further as a pregnancy specific hormone.

Among other agonadal epithelium it is also expressed in the endometrium of the non-pregnant women with a maximum at the time of implantation windows. In case of a pregnancy this effect is intensified in the decidua as we have demonstrated in previous studies. The endometrial hCG could be a key marker for receptivity.

T-065 Implantation

Reduced pregnancy rates – another factor to decrease live birth rates in ART in women with uterine anomalies

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In a retrospective matched case-control study we recently published data showing that the abortion rate before hysteroscopic metroplasty was significantly higher, both in women with a small partial septum (78.9% before resection vs 23.7% in the normal controls, OR 12.08) and a large septum (83.3% before resection vs 16.7% in normal controls, OR 25.00) compared to women with a normal uterus. After the surgery, the abortion rate was comparable to the abortion rate in women with normal uteri: in both women with a small partial and women with a larger septum. The aim of the present retrospective matched case-control study was to evaluate the effect of septate, subseptate and arcuate uterus on pregnancy rates after 2481 ETs in conventionally stimulated IVF/ICSI cycles. The study group consisted of 289 ETs before and 538 ETs following hysteroscopic resection of the uterine septum. For each ET in the study group, we found 2 consecutive ETs in women without uterine anomalies. The study and the control groups were matched by age, BMI, stimulation protocol, embryo quality, IVF or ICSI, and infertility etiologies. The number of embryos transferred, embryo quality and absence of uterine anomalies significantly predicted the pregnancy rates in the study group; OR: 1.6, 2.6, and 2.5, respectively (p < 0.001). The pregnancy rates after ET in the group of women before hysteroscopic resection were significantly lower, both in women with subseptate or septate uterus (OR 2.9, p < 0.001), and in women with arcuate uterus (OR 2.1, p < 0.002) than in women with normal uteri. The difference was even more evident if 2–3 embryos with at least 1 best-quality embryo were transferred: OR 7.3; p < 0.001 and 2.1; p < 0.03, respectively. After surgery, the pregnancy rates in the study groups were comparable to the controls.

As expected, the live birth rates after a transfer of 1–3 embryos regardless the embryo quality before hysteroscopic resection were significantly lower, both in women with subseptate or septate – 2.6% vs 21.7% uterus (OR 10.15; p < 0.001) as in women with small partial uterine septum vs arcuate abnormality 2.8% vs 21.3% (OR 9.7; p < 0.001).

T-066 Implantation

Resistance index S/D of uterine blood flow has a predictable value for successful embryo implantation

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Introduction During controlled ovarian hyperstimulation for IVF, the level of steroid hormones (estrogens and progesterone) are changing dramatically and lead to rapidly changing of endometrium and uterine vascularity, which inevitable can influence some Doppler parameters. Because of the discrepancies at the literature we aimed to evaluate the uterine blood supply by Doppler sonography, which could give more particular details of the uterine receptivity and consequently more tools for better management of the most important stage of performed in-vitro fertilization (IVF) procedure – an embryo implantation process.

Materials and Methods A prospective, multi-centric study was performed with mean age 32.9 (SD ± 5.4), who were implemented in IVF cycles. We carried out serial Doppler ultrasound assessment of ascending branches of a uterine at the day of hCG and at the day of embryo transfer (ET). Waveform analyses, observation of diastolic notch and estimation of definite resistance indices (PI, RI, S/D) were performed as well as variables related to patient’s clinical characteristics, treatment characteristics, ovarian response, ovum retrieval, outcome of IVF, embryo transfer in terms of outlining of a group with equal including criteria. The received results were compared between pregnant and non-pregnant women. Statistical analyses were performed using descriptive analysis and t-test/Student’s test.

Results In this study we found out significant declining of S/D from hCG-day to ET-day at pregnant group (13.3 to 4.04; p = 0.03), whereas in non-conceived group observed tendency of increasing of S/D, but without significance (8.3 to 10.2; p = 0.29). Moreover,
after dividing of pregnant group to ongoing pregnancy and miscarriages we received the same result only at ongoing pregnancy (p = 0.03), while in miscarriage group declining of S/D was non-significant (p = 0.13). Also we detected significant difference of S/D between pregnant and non-pregnant women at the day of ET (4.04 vs 10.2; p = 0.05). No other statistically significant results were recorded.

Conclusions This study demonstrates significant declining of S/D parameters during the peri-implantation period in ongoing pregnancy group in contrast to non pregnant and miscarriages. Our results rise the assumption of predictable value of S/D = 4.04 (SD ± 2.33) at the day of ET for receptive endometrium and successful implantation.

T-067 Implantation
The effect of endometrial thickness on IVF/ICSI outcome

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Introduction During the menstrual cycle the endometrium undergoes cyclic changes in preparation for implantation. If this preparation is not sufficient, then implantation will fail. The impact of endometrial thickness on the day of embryo transfer on IVF outcome was investigated in the present study.

Patients and Methods A total 414 cycles of IVF were studied We have gathered for every patient: the type of performed ART, the age, the length of barrenness, the reason of barrenness, the number of the previous performed attempts, hormonal status before the beginning of the IVF attempt (FSH, LH, prolactin, estradiol), the protocol of ovarian stimulation used (long agonist, short agonist or antagonist), the type of gonadotrophin used (hMG or re-combiant FSH), endometrial thickness the day of the induction of ovulation and finally the success of a pregnancy.

Results and Discussion The endometrial thickness after ovarian stimulation was significantly linked to the rate of FSH before the IVF attempt (p = 0.01). The total rate of pregnancies (23%) was significantly linked to the endometrial thickness before the ovulation induction (p = 0.02). For patients between the age of 30 and 34 year old, there was a statistically significant relation-ship between the rate of pregnancies and endometrial thickness. Endometrial thickness ≥ 12 mm was associated with the highest IVF success rate of 54 %.

There was a statistically significant relationship between the endometrial thickness and the stimulation protocol used (p < 0.001). The long agonist protocol results in an endometrial thickness ≥ 12 mm; while the short agonist protocol results in an endometrial thickness < 7 mm.

Conclusion The results of the present study identified a statistically significant difference mean endometrial thickness between cycles that resulted in pregnancy and those that did not. Adequate endometrial development is required for pregnancy to occur, and pregnancy rates were found to be higher when the endometrium reached at least 12 mm thickness. Consequently, clinicians providing IVF for infertile couples must pay close attention to endometrial development as well as to follicle growth.

T-068 Implantation
Decidualized HESCs treated with hCG become resistant to cell death induced by oxidative stress

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Purpose Human chorionic gonadotropin (hCG) is one of the earliest embryonal signals secreted by embryo. In addition to its well-known luteotropic function at the onset of pregnancy, it appears to modulate endometrium in preparation for implantation. However, the precise effects of hCG for decidualization process are poorly understood. Recently, we demonstrated that human endometrial stromal cells (HESCs) became extraordinarily resistant to oxidative stress-induced apoptosis upon decidualization. Therefore, we hypothesized that the direct action of hCG on HESCs decidualization might also induce some changes in gene expression that would result in resistance of oxidative stress and support implantation. Here we investigated that recombinant hCG (r-hCG) has an effect on morphological and biological changes for decidualization of HESCs in vitro.

Methods and Materials Primary HESCs cultures were established, propagated, and confluent cultures were decidualized with 8-h-cAMP (0.5 mM) and MPA (10−5 M) and with or without various concentrations of rhCG for 4 days. Hydrogen peroxide (H2O2) treatment was used as a source of ROS. The amounts of PRL in culture media were examined. Western blot was performed for protein analysis. The level of apoptosis was analyzed by cell detection ELISA kit.

Results Treatment with 8-Br-cAMP plus MPA pronounced stimulated PRL secretion which is known as a decidualizing marker. Addition of r-hCG at various concentrations to decidualized HESCs caused a dose-dependent decrease of PRL production. Decidualized HESCs treated with rhCG dose-dependently decreased cell death induced by oxidative stress. Although rhCG treatment on its own did not elevate the expression of FOXO1 on non-decidualized HESCs, rhCG dose-dependently further augmented MPA/cAMP induced FOXO1 and Mn-SOD expression, but not Cu, Zn-SOD and Catalase. Recombinant hCG also inhibited the expression of the pro-apoptotic Bax protein and up-regulated anti-apoptotic Bcl-2 levels in decidualized HESCs exposed to ROS.

Conclusion These results indicate that hCG-treated decidualized HESCs expressed FOXO1 and Mn-SOD and acquired resistance to oxidative stress, suggesting that hCG may improve the uterine environment for successful implantation.

T-069 Implantation
Genetic markers for thrombophilia in patients with unexplained infertility in Mexican population

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Introduction When unexplained infertility exists, ethiopathologic study is delayed. Genetic mutations, which predispose to thrombophilia, must be determined as these may interrupt implantation and trophoblastic development.

Objective To describe the incidence of genetic markers for thrombophilia in patients with Unexplained Infertility in Mexican Population.

Materials and Methods Genetic markers for thrombophilia were determined in 20 women with unexplained infertility. Numerical and categorical variables were determined as median and interquartile range, represented in frequencies and percentages.

Results The mean age was 34 (32–37) years. Positive markers were reported in 95%, with 85% carrying two or more mutations. The most frequent mutation was for MTHFR, present in 90%, followed by PAI 1 in 80%, Factor XIII in 40% and HPA-1 in 20%.

Conclusions The reported correlation between genetic markers for thrombophilia and unexplained infertility is 20–68%. Our results show that the frequency and type of mutation differs from what has been previously published for other study groups, being of 95% for the Mexican Population. The causal relationship is strong enough to suggest the necessity for early diagnostic measures, more controlled studies and therapeutic options.
T-070 Implantation

Endometrial cell proliferation and vascularization changes in women with reproductive failures?

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Introduction

Reproductive failure, determined as recurrent implantation failure (RIF) in assisted reproduction, as well as recurrent spontaneous abortions (RSA) is not well understood. Several factors including low quality embryos, and cellular and molecular changes in endometrium may contribute to the insufficient fetal-maternal interaction resulting in pregnancy failure. Prior clinical studies suggest an inadequate endometrial growth and development of the endometrium, leading to a lesser endometrial thickness. We therefore aimed to determine the cellular proliferation and expression of markers of vascularization such as factor VIII (a marker of blood vessels), but Ki67 was detected in cells of endothelial cells and smooth muscle cell actin (SMCA; a marker of pericytes and smooth muscle cells) in endometrium of women with reproductive failure compared to healthy controls.

Materials and Methods

During the luteal phase (LH+8/9) endometrial tissues were collected after informed consent from 5 controls and 10 women with reproductive failure and immediately frozen. Serum was collected for hormone analysis. Immunohistochemistry was used to determine labeling index (proportion of proliferating Ki67-positive cells out of total cells per tissue area) and expression of factor VIII and SMCA followed by image analysis.

Results

Concentration of estradiol-17β and progesterone was similar among groups. Factor VIII, SMCA and Ki67 were detected in endometrial tissue sections for all groups. Factor VIII and SMCA were localized to blood vessels, but Ki67 was detected in cells of uterine glands, stromal tissue and blood vessels. Cellular proliferation but not expression of factor VIII or SMCA was decreased in endometrium of women with RIF, as well as with RSA compared to healthy controls.

Conclusion

Thus, our data suggests that reproductive failure is associated with insufficient cell proliferation/tissue growth, while there is no difference in early endometrial vascularization. As hormone levels were not found to be significantly different, additional studies are required to determine expression of factors controlling tissue growth and cell proliferation in these pathological conditions.

T-071 Implantation

Influence of endometrium on cytotrophoblast gene expression: Interaction of endometrial stromal cells and first trimester trophoblast in a co-culture system

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Introduction

During the invasion of trophoblast into the maternal endometrium, multiple interactions take place that are nearly impossible to analyze in vivo. In an in vitro system we analyzed the influence of endometrium on trophoblast.

Materials and Methods

First trimester trophoblast explants were cultured in six different conditions: together with non-decidualized stromal cells, with decidualized stromal cells, with conditioned medium (CM) from non-decidualized stromal cells and from decidualized stromal cells, CM from first trimester decidua and on collagen coated plastic. After 48 h the outgrown cytotrophoblast cells were isolated and RNA was extracted. Gene expression of Hypoxia responsive factor-1, IGFBP-3, IGF-II, INSL-4, LOX, HSD1B2 and glycolerin was analyzed by real time PCR and protein production by western blot analysis.

Results

Of the analyzed genes a significant up-regulation of glycolerin mRNA in cytotrophoblast cells co-cultured with decidualized endometrial stromal cells as well as in cytotrophoblast cells co-cultured with CM of pregnancy decidua was detected. This data was validated on the protein level. No significant change was found in any of the other analyzed genes.

Conclusions

A direct influence of endometrium/decidua on first trimester cytotrophoblast is shown for the first time. Even though all analyzed genes have previously been shown to be important in the fetal-maternal interface, only glycolerin was significantly regulated by endometrial stromal cells and decidua in cytotrophoblast cells.

T-072 Implantation

Endometrial hCG priming in IVF-therapy

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Introduction

The establishment of pregnancy requires an interaction between the embryo, the uterus and the corpus luteum. Successful implantation requires a genetically normal Embryo and a receptive uterine endometrium. Human chorion gonadotropin (hCG) was long thought to be solely the trigger for progesterone production by the corpus luteum. However, recent publications suggest that hCG plays an important role in angiogenesis and in maintenance of cell proliferation. Moreover, it was shown that hCG regulates the uterine receptivity by the endometrial competence to nourish the implanting embryo. During IVF-therapy successful implantation is still a challenge for physicians and embryologists. We developed a method for priming the endometrium before transfer of embryos during IVF-therapy to improve the implantation rate.

Methods

52 patients were grouped into three groups. First group A consisted of 18 patients treated with 500IU hCG (Pregnyl, Organon) After the end of oocyte retrieval the hCG was solved in IVF-embryo transfer media (Cook, Australia) and infused with a insemination catheter in a volume of 200 µl. Group B consisted of 18 patients treated in the same way with 100 IU hCG. Group C consisting of 16 patients was treated with the solvent solution alone (control). After an additional 30 min ± 10 min waiting time patients could leave the institute. Patients suffering from ovarian hyperstimulation syndrome (OHSS) in previous IVF attempts were excluded from the study.

Results

The method is safe and no complications were observed. From a laboratory point of view it is easy to prepare the additional medications and to load the second catheter for endometrial priming. For the patients an extra-time of approximately 15 min ± 7 were used until they can leave the operating theatre. Median age at the time-point of oocyte recovery was 37.6, 37.3 and 37.8 in groups A, B and C respectively. In our setting the implantation rate determined by biochemical pregnancy and the pregnancy rates after 6 week of gestation were highest in the group treated with 500IU hCG and lowest in the control group without hCG infusion.

Conclusion

In our institution endometrial hCG priming during IVF-therapy increases both implantation as well as pregnancy rates. However, further studies including more patients are necessary to extend the patient number in each group and confirm our results.

T-073 Infections

A survey of the prevalence of vaginitis and vaginosis common microbial factors in outpatients and bedridden patients in north of Iran during 2007–2008

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Vaginitis is among the most common conditions for which women seek medical care, with vaginal discharge accounting for 10 million office visits each year. Although vaginitis can have a variety of causes, it most often is associated with infection or atrophic changes. Common infectious forms of vaginitis include bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis. Although these infections generally respond
Urinary tract infection is defined as significant bacteriuria in the presence of symptoms. This common clinical entity accounts for a significant number of emergency department visits. It is estimated that about 20% of women are affected at least once during their lifetimes. The goal of this study has been the determination of prevalence of urinary tract bacterial infections among female students in universities of Guilan province in north of Iran. Out of 207 cases 32 (15.46%) were found to have urinary infections. The study sample was in 18–29 age groups. Out of total cases, 18 (8.7%) were suspected to have UTI. After re-testing these cases, 3 (1.45%) were positive. It was also found that the most positive cases were among 18–21 age groups and the least positive cases were among 26–29 age groups. By applying Chi-square test and due to limited age of study among 26–29 age groups, there was no significant relation between increasing of age and positive cases. Out of 3 cases, 19 (59.37%) had asymptomatic bacteriuria and 13 cases (40.63%) had symptoms. There was significant relation between asymptomatic and symptomatic bacteriuria considering cultured positive bacteriuria (df = 1, Chi-square = 84; p = 5%).

**Main Source of Funding** Islamic Azad University of Iran.

**T-074 Infections**

**Study of urinary tract infections in Iranian female students in universities of Guilan Province**

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Urinary tract infection is defined as significant bacteriuria in the presence of symptoms. This common clinical entity accounts for a significant number of emergency department visits. It is estimated that about 20% of women are affected at least once during their lifetimes. The goal of this study has been the determination of prevalence of urinary tract bacterial infections among female students in universities of Guilan province in north of Iran. Out of 207 cases 32 (15.46%) were found to have urinary infections. The study sample was in 18–29 age groups. Out of total cases, 18 (8.7%) were suspected to have UTI. After re-testing these cases, 3 (1.45%) were positive. It was also found that the most positive cases were among 18–21 age groups and the least positive cases were among 26–29 age groups. By applying Chi-square test and due to limited age of study among 26–29 age groups, there was no significant relation between increasing of age and positive cases. Out of 3 cases, 19 (59.37%) had asymptomatic bacteriuria and 13 cases (40.63%) had symptoms. There was significant relation between asymptomatic and symptomatic bacteriuria considering cultured positive bacteriuria (df = 1, Chi-square = 84; p = 5%).

**Sponsor** Islamic Azad University of Iran-Lahijan branch.

**T-075 Infections**

**Pseudomonas aeruginosa in cases of infertility: Biofilm formation and effect on early cleavage embryos**

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**Pseudomonas aeruginosa** from pure sperm preparation of infertile men with good sperm concentration used in the insemination of IVF culture produces biofilm infection on day 3 in embryo culture dish. The cleavage embryos were found to be arrested upon the formation of the exopolysaccharide.

**T-076 Infections**

**Prevention of HPV reinfection with vaccination after Laser Vapourisation and Conisation in reproductive age patients with HSIL (open controlled study)**

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**Objectives** Prevention of HPV reinfection by “Gardasil” after surgical treatment of patients with high grade intraepithelial lesion HSIL-CIN 2 and Human papilloma virus (HPV) infection.

**Methods** 145 patients with HSIL-CIN 2 were investigated (Pap smear, colposcopy, biopsy, immunohistochemistry P16 + PCR). All investigated patients (n = 145) with HSIL-CIN 2 were treated by Co2 Laser Conisation and vapourisation. Vaccination by “Gardasil” was recommended. I study group includes 53 patients who agreed vaccination. They got “Gardasil” after surgical procedure and before having sex. II study group includes 92 nonvaccinated patients. In both groups control PAP smear, colposcopy and PCR detection of HPV (type – 6, 11, 16, 18, 31) infection after surgical treatment with 3 month intervals during one year were carried out.

**Results** No HPV induced lesion during one year was revealed I study group (PCR HPV). In 2 study groups there were found cases of HPV induced lesion: After 3 months: In 5.6% by colposcopy: TZ, acetowhite epithelium, flat condyloma. In Pap smear HPV induced lesions were not revealed, PCR (16, 18) were positive. After 6 months: In 7.5% by colposcopy: TZ, acetowhite epithelium. In Pap smear HPV induced lesions were not revealed, PCR (16, 18) were positive. After 9 months: In 11.3% by colposcopy: TZ, acetowhite epithelium, fine punctuation. In Pap smear LSIL-CINI-HPV, PCR (16, 18) were positive. After 12 months: In 22.6% by colposcopy: TZ, acetowhite epithelium, puation, mosaic. In Pap smear LSIL-CINI-HPV, PCR (16, 18) were positive. HPV induced lesion was statistically significant at 6, 9 and 12 months (p < 0.05).

**Conclusions** Due to preliminary data we are able to suppose, that vaccination by “Gardasil” after laser surgery of intraepithelial lesion may prevent HPV reinfection.

**T-077 Infections**

**Chlamydia trachomatis infection in patients from an academic tertiary infertility care**

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**Introduction** Chlamydia trachomatis (C. trachomatis) is the major cause of mucopurulent cervicitis; but unfortunately roughly 70%–75% of infections are asymptomatic. The sharp worldwide increase in the incidence of inflammatory pelvic disease during the last two to three decades has led to an epidemic character secondary to tubal factor in both primary and secondary infertility.

**Objective** To evaluate the prevalence of C. trachomatis infection and its relation to the types of infertility and to the changes found on hysterosalpingogram in patients from an academic tertiary infertility care.

**Methods** Retrospective analysis of medical records of 98 patients treated at the outpatient infertility clinic at the Universidade de Caxias do Sul, from January 2007 to July 2009.

**Results** Only 39.2% of the patients tested negative for Chlamydia trachomatis. Active infection was diagnosed in 30.4% of patients, and the others presented with reagent titles suggestive of prior infection. When correlated the presence of active C. trachomatis infection and its type of infertility, similar frequencies was observed for both primary and secondary infertility: 29.7% and 31.0%, respectively. In addition, 32.4% with primary infertility and 28.6% with secondary infertility had reagent titles suggestive of prior infection. Still regarding to serum tests for C. trachomatis, it was found that among the 50 abnormal hysterosalpingogram results, 47 (94.0%) were related to positive tests – 31.9% of patients with prior infection and 36.1% of patients with active infection. Hidrosalpinx and tubal obstructions (not related to tubal ligation) was present in 88.9% and 62.1% of cases of patients tested positive for C. trachomatis, respectively.

**Conclusion** The C. trachomatis infection, prior or active, showed a high prevalence among the patients analyzed in this study, and it was the main cause of tubal abnormalities detected upon hysterosalpingogram. This data draw attention to the importance of...
sexual transmitted diseases prevention and suggests that the pelvic inflammatory disease caused by *C. trachomatis* is the most important preventable cause of infertility.

**T-078 Infections**

**Infertility management at 2 medical centers in Kinshasa**

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**Objectives**

To determine the findings at the infertility investigations and treatment in a sub-Saharan infertility population.

**Materials and Methods**

Between January 1999 and December 2007, a total of 1923 patients sought for infertility care in 2 medical centers. The findings in anamnesis, clinical examinations, diagnosis investigations and treatment were analyzed.

**Results**

The average age of the couples was 32 ± 5.5 years old for the woman and 39 ± 5.3 years for the man; 2% of them are great multiparous (> 4 children); 64% had secondary infertility; the average infertility duration before seeking care was 4.5 ± 3.7years. Main diagnoses after preliminary investigations were pelvic inflammatory disease (49%) especially among teenagers (80%); uterus myomata (24%) and reproductive endocrinopathy (polycystic ovary; hyperprolactinemia) (18%).

The basal investigations showed that: the post-coital test (n = 415) was pathologic in 85% of the patients; at hysterosalpingogram (n = 571): tubal occlusion (53%), uterus synchiae (8%); at endometrial biopsy (n = 309): tuberculosis endometriti (1,3%), non-specific endometriti (11%) and retained fetal bone (0,7%); at spermogram: azoospermia (4%), oligozoospermia (asthenozoospermia) (78%). A quarter of the patients abandoned infertility investigation and treatment after 6 days and half of them after 45 days. 216 (11,2%) became pregnant; half of them after 5 months.

**Conclusion**

Infertility patients sought of care more and more old (in their thirties). Infertility management is dominated by pelvic infections and its consequences. Reproductive endocrinopathy begin to gain in importance in this population. Treatment adhesion is very low in this population.

**T-079 Infections**

**Bee propolis as a new modality for treatment of H1N1 influenza virus in pregnant women**

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**Results**

The average age of the couples was 32 ± 5.5 years old for the woman and 39 ± 5.3 years for the man; 2% of them are great multiparous (> 4 children); 64% had secondary infertility; the average infertility duration before seeking care was 4.5 ± 3.7 years. Main diagnoses after preliminary investigations were pelvic inflammatory disease (49%) especially among teenagers (80%); uterus myomata (24%) and reproductive endocrinopathy (polycystic ovary; hyperprolactinemia) (18%).

The basal investigations showed that: the post-coital test (n = 415) was pathologic in 85% of the patients; at hysterosalpingogram (n = 571): tubal occlusion (53%), uterus synchiae (8%); at endometrial biopsy (n = 309): tuberculosis endometriti (1.3%), non-specific endometriti (11%) and retained fetal bone (0.7%); at spermogram: azoospermia (4%), oligozoospermia (asthenozoospermia) (78%). A quarter of the patients abandoned infertility investigation and treatment after 6 days and half of them after 45 days. 216 (11.2%) became pregnant; half of them after 5 months.

**Conclusion**

Infertility patients sought of care more and more old (in their thirties). Infertility management is dominated by pelvic infections and its consequences. Reproductive endocrinopathy begin to gain in importance in this population. Treatment adhesion is very low in this population.

**T-080 Infections**

**Cytokines at the genital chlamidiosis**

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**Objectives**

The aim of our research was to study IL-10, IL-12, IL-17 and IL-23 in the mentioned processes. For this aim we have investigated 473 patients with having the impairment of the genital system (176 men and 297 women, age 16-62 years) infected with chlamidia (Ch. trachomatis). 82 among them were primarily infected, 391 were chronic carriers of chlamidia.

In the control group there were included 37 practically healthy patients and 64 non-chlamidia infected probands having other genital infections.

**Methods**

The interleukins study was carried out on the biological materials of the blood and impairment areas using the IFA, IFU and PCR methods. The genital infections diagnostics was carried out by the PCR.

**Results**

By statistically elaborating the results it was stated that the patients having the high level of IL-10 were distinguished with the prolonged forms of the disease (p < 0.001; r = 0.38), and in the probands infected primarily with high IL-12 it was as follows: 42 patients out of 82 (p < 0.005; r = 0.68). IL-17 was increased with the patients having acute (p < 0.005; r = 0.57) and primary chlamidia (p < 0.001; r = 0.64).

The existence of IL-23 was stated in the majority of the patients but creditably high it was just in the patients having auto-immune processes (p < 0.001; r = 0.41).

**Conclusion**

According to the obtained results we can conclude that IL-10 and IL-23 must be participating in the Chlamidiosis chronization. As to IL-12 and IL-17 they appear as the mediators of acute processes. IL-13 must not be active in the immune-pathogenesis of Chlamidiosis.

**T-081 Infections**

**Seminal plasma protects Candida albicans from phagocytosis by dendritic cells**

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The molecular basis of the interaction of commensal or pathogenic microbes of the genitourinary tract with human spermatozoa has only received little attention so far. Nevertheless, infertility in men and women frequently correlates with microbial contamination, and seminal plasma functions potentially not only as a vehicle for sperm, but also as a carrier for pathogens. Therefore, we set out not only to monitor potential adverse effects of *Candida albicans* on spermatozoa, but also studied the influence of seminal fluid on host pathogen interaction. Since the yeast *C. albicans* as well as bacterial pathogens are known to release distinct signaling molecules in substantial amounts, we investigated whether such molecules can directly affect human spermatozoa.

We found that the *C. albicans* quorum-sensing molecule farnesol and the *Pseudomonas aeruginosa* 3-oxododecanoyl-L-homoserine lactone, respectively, induce multiple damages in human spermatozoa. A reduction in the motility of spermatozoa coincided dose-dependently with apoptosis and necrosis at concentrations which were non-deleterious for dendritic-like immune cells. More interestingly, sublethal doses of both signaling molecules induced premature loss of the sperm acrosome, a structure which is essential for fertilization. Investigating the interaction of either sperm or *C. albicans* with phagocytes of the reproductive tract we discovered that seminal plasma protects not only sperm from phagocytosis by immature dendritic cells, but also the fungal cells. This phenotype was not attributed to a cytotoxic effect of seminal plasma on dendritic cells, but to some extent to an induced maturation of these phagocytes.

Our findings uncover a new facet in the interaction of microorganisms with human gametes, give further insights into the interaction of spermatozoa with dendritic cells, and
suggest a potential immune evasion strategy for microbial pathogens.

**T-082 Infections**

**Mixed chlamidal infection and female infertility**

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**Aim** Detection of isolated and mixed chlamidal infection in females with various forms of infertility.

**Materials and Methods** 578 females (18–39 years group) with various forms of infertility were investigated. In all patients, clinical, laboratory, ultrasonographic, hormonal and X-Ray investigations and in 217 patients diagnostic laparoscopy was carried out. Bacterioscopic, bacteriological, immunofluorim and PCR diagnostics were used for STIs detection.

**Results** The frequency of urogenital chlamidal infection was significantly higher in infertile females compared to fertile (57.3% and 11 % correspondently). It was the highest in the group of females with fallopian tubes occlusion (84.6%) and was significantly higher as compared to frequency of infertile female group with normal tubes (15.4%). In infertile females urogenital chlamidiosis mostly revealed by mixed infections (mixed chlamidal infections 85.7%, isolated 16.3%). The highest rate association of chlamidiosis was noticed with trichomoniasis (63.7%) and ureaplasmosis (36.3%). By PCR method in common group of infertile females urogenital chlamidiosis in cervix samples were detected in 15.2%. In patients with tuboperitoneal infertility samples were obtained from Douglas cavity by laparoscopy and urogenital chlamidiosis were detected in 62.6% of cases, in 42.6% it was associated with ureaplasmosis and gonococcal infection. Above mentioned indicates about importance of urogenital chlamidiosis, especially its mixed forms in tuboperitoneal infertility.

**Conclusion** Early detection of chlamidal infection, particularly its mixed forms, in infertile females is very important for avoidance of fallopian tubes irreversible occlusion. Correct diagnostics at early stages is main denominator for comprehensive and effective treatment. In fertile females before intrauterinal invasive procedures investigation for chlamidiosis and other STIs is essential for avoidance of ascending infection, while they are the main cause of fallopian tubes occlusion.

**T-083 Infections**

**The correlation of chronic Chlamydia trachomatis infection and pelvic adhesions identified by laparoscopy. A clinical study of 135 cases**

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**Aim** To investigate the correlation of chronic infection with Chlamydia trachomatis, and pelvic adhesion syndrome as a cause for infertility.

**Materials and Methods** During a 2 year period (2008–2009) 135 infertile patients with complete evaluation including Chlamydia trachomatis seric IgG for Elisa method. All patients had been approached by hysteroscopy and laparoscopy with dye test.

**Results** Our patients were aged 25–41 years. Comparing the positivity of IgG with the presence of pelvic adhesions, we obtained a sensitivity 48.48%, specificity of 95.45%, positive predictive value 91.42%, negative predictive value 64.94%.

**Conclusions** When IgG for Chlamydia is positive in an infertile patient, there is an indication for a laparoscopy to investigate the presence of pelvic adhesions. But negative IgG for Chlamydia does not exclude pelvic adhesions, which could be produced by other causes: endometriosis, previous pelvic surgery, abortions, IUD, etc.

**T-084 Infections**

**Study of the reasons of infectious abortions in the subjects who approached Kamali Hospital, Karaj in Iran**

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**Research Background and Objective** Infectious diseases are one of remarkable problems in women such that 15% of women’s death rate results from abortion. Almost half of this rate is caused by infections. The subjects in Kamali Hospital in Karaj were studied considering the parameters of age, pregnancy term, abortion history and contamination by infectious factors that the findings can be used in hygienic planning.

**Methods** Serological tests of ELISA and IFA were conducted on serums obtained from 84 subjects’ blood and vaginal secretion was also used for culture in some cases.

**Results** Of 84 samples collected due to Toxoplasmosis, Mycoplasma, Listeria, Brucella and Chlamydia infectious factors from July–August 2006 to February–March 2007, 17 samples (20%) were related to Toxoplasmosis, 30 samples (35%) were related to Mycoplasma infection, 9 samples (11%) were related to Listeria infection, 8 subjects (9.5%) were related to Brucella infection and 2 samples (2.3%) were related to Chlamydia infection and the highest rate of abortion occurred in 20–30 ages and the highest rate of frequent abortions has occurred in 20–30 ages as well.

**Discussion** Considering importance of infectious factors which result in abortions, we concluded that the most incidences are related to infectious factors of Mycoplasma, Toxoplasma and Listeria. Considering zoootics of Toxoplasma and Listeria factors and this that Karaj is a region where people are engaged in animal husbandry, we may find a relation between abortion and these factors. Mycoplasma is also spread by sexual relations and considering the fact that the age groups who abort are young, thus it is necessary to study infectious factors, age parameters, pregnancy term and frequent abortions in Karaj.

**T-085 Infections**

**Comparison of tubal pathology induced by transvaginal and intraperitoneal inoculation with Chlamydia trachomatis in mice**

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**Objectives** Chlamydia trachomatis (Ct) induces many aspects of clinical condition, such as cervicitis, pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility. However, the pathogenesis of Ct infection is not still clarified yet. We established a mouse model for tubal factor infertility induced by transvaginal Ct infection. In this study, Ct was intraperitoneally inoculated in mice to compare the severity of tubal and pelvic infection with transvaginal inoculation.

**Materials and Methods** 5 weeks old female BALB/c mice were prepared with 2.5 mg of medroxyprogesterone acetate. Seven days later, mice were transvaginally and intraperitoneally inoculated with 10^7 inclusion-forming units (IFU) of the Chlamydia muridarum (Cm). The control mice were administered sucrose-phosphatic buffer with same way of inoculation. The oviducts were removed 35 days after the initial inoculation with Cm. The histopathological diagnosis was obtained by the hematoxylin and eosin (HE) staining, using a light microscope.

**Results** Tubal epithelial damage by intrauterine Ct inoculation was also histologically confirmed in mice. Tubal damage, such as extensive destruction of the ciliary epithelium, interstitial thinning, enlargement of the cavity, increased secretion in the oviduct and epithelial exfoliation, in mice inoculated intraperitoneally were more severe than that in mice inoculated transvaginally.
Interestingly, there were no marked histo-
logical changes on the serous membrane sur-
face in the mouse oviducts intraperitoneally
inoculated, which were not also found in
those inoculated intravaginally.

Conclusions A novel mouse model for tu-
bital infertility was developed in the present
study. Although the specific tubal epithelial
response to Ct infection was suggested, fur-
ther studies are required to prove how tubal
pathogenesis is immunologically regulated.

T-087 Infections
Genital infection and infertility – is there a role for screening all STD? A case-control study
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Background Genital infection is one of the most important causes of infertility, affecting:
falllopian tubes, endometrial mucosa, sperm parameters.

Methods We analyzed the presence of Chlamydia Trachomatis (CT) Antigen, Ureaplasma Urealyticum (UU), Mycoplas-
ma Hominis (MH) and Neisseria Gonor-
rhoeae (NG) in the endocervical secretions and Chlamydia Trachomatis Antibodies IgA, IgG, IgM in the blood of 111 infertile
women as well as in 30 pregnant women in the 3-rd trimester of pregnancy, as a control
group.

Results 47.74% of infertile women had at least one of the previous infections. Only
1.8% were positive for CT Antigen, but
15.31% were positive for CT IgG, 4.5% for
CT IgM. 1.8% for CT IgA, 0.9% were posi-
tive for NG, 3.6% for MH and 36% for UU.

From the control group, none were positive
for CT Ag but 3.33% for CT IgA, 0% for CT
IgM and 1.8% for CT IgG. None was posi-
tive for NG, but 26.66% were positive for
MH and 63.33% for UU.

Conclusions
1. We could not prove any association be-
tween genital MH and UU and infertility as
the prevalence was higher in the con-
trol group than in the cases, but the corre-
lation of the CT infection with the infertil-
ity was clearly shown.
2. However, it is necessary to perform rou-
tine tests to screen for CT, NG, UU and
MH among infertile patients.
3. The positivity for CT IgG is a marker bet-
ter correlated with fallopian tube obstruction
than the CT antigen.

T-088 Infections
Aspartate aminotransferase test in vaginal washing fluid for the detection of preterm premature rupture of membranes
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Background PPROM refers to rupture of the fetal membranes before the onset of labor at
less than 37 weeks gestation. It complic-
ates only 2–3% of pregnancies but is associ-
ated with 40% of preterm deliveries and can result in significant neonatal morbidity and
mortality. False diagnosis of PPROM may lead to limit unnecessary obstetric interven-
tions, including hospitalization, administra-
tion of antibiotics and corticosteroids, and
even induction of labor, while delayed diag-
nosis of PPROM may worsen the adverse
consequences that may complicate PPROM.

Aim The aim of the current study was to de-
termine whether measurement of AST and
ALT levels in vaginal fluid is useful for the
diagnosis of preterm premature rupture of
membranes.

Patients and Methods Case control study of a total 90 pregnant women were in-
cluded in the study between 26 and 36 weeks of
gestation divided into two groups 45 preg-
nant women with PPROM were included as
the study group. Further 45 pregnant women
without PPROM, matching the women of the
study group, were included as a control
group. Vaginal fluid AST and ALT level were
measured in both groups.

Results Vaginal fluid AST concentration
was significantly higher in women with
PPROM compared to women of the control
group 18 (11–34) IU/L vs 0 (0–3) IU/L,
respectively; p < 0.0001. Vaginal fluid ALT con-
centration was significantly higher in
women with PPROM compared to women of
the control group 4 (1–10) IU/L vs 0 (0–1) IU/
L; p < 0.0001, respectively. The best cut-off
value for vaginal AST concentration for de-
tection of PPROM was 1.25 IU/L gave a sen-
sitivity 97.8%, specificity 62.2%, PPV
72.3%, NPV 96.55%. The best cut-off value
for vaginal ALT concentration for detection
of PPROM was 0.5 IU/L gave a sensitivity
86.7% specificity 75.6%, positive predictive
value 78% and negative predictive value
85%.

Conclusion Measurement of AST and
ALT in vaginal washing fluid is a cheaper,
faster and reliable method for detection of
preterm prelabor rupture of membranes, it
has a high sensitivity and negative predictive
values so it is considered a good screening
test for detection of PPROM.

T-089 Infertility diagnosis
The outcomes of pregnancy in different ways of conception in the infertility women
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Introduction The WHO shows, that every
8th family faces a problem of conception, and
every 6th matrimonial pair is infertility. We
aimed to examine cases, duration and an out-
come of pregnancy woman’s suffered
against infertility with different ways of con-
ception.

Materials and Methods This cross-sec-
tional study involved 457 infertilities women
(mean age 33.6 ± 4.4 years). All patients
had been divided in two groups: 1st group: in
vitro fertilization (IVF) (n = 110), mean age
33.45 ± 0.44 years; 2nd group: natural fertili-
ze (NF) (n = 347), mean age 30.6 ± 0.47
years. The anamnesis, hormonal state, clini-
cal states of newborns (Apgar, Petrusso
score) were measured in both groups. All the

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analyses were performed with the Statistics 6.0 software. p-value < 0.05 was accepting as statistically significant.

**Results** By the analyzing anamnesis high percent of appendectomy in the childhood was established. In 1st group mean BMI (30.26 ± 0.6 kg/m²) was defined as obesity, and in 2nd (25 ± 0.5 kg/m²) as overweight. The main reason of development infertility in both groups was tuboperitoneal factor. In NF group prolactinemia was revealed more often, than in IVF (p < 0.05). Among complications of pregnancy in 1st group significantly more often threat of miscarriage and gestosis were revealed, while in NF gestational diabetes is more often (p < 0.05). The analysis of partum ways: in IVF group partus caesarius; in NF group mainly through vaginal ways significantly (p = 0.05) was more often spent. A condition of newbornds significantly differed on indicators score Apgar, Petrusso, anthropometric indicators state in IVF group children were functional immature.

**Conclusions** The high percentage in the anamnesis appendectomy and obesity were revealed. In women’s subjects after IVF pregnancy significantly became complicated threat of miscarriage and gestosis. Newborns in IVF group were more functional immature.

**T-090 Infertility diagnosis**

**Overcoming infertility by ZIFT and IVF: A clinical case study**

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IVF and ZIFT are 2 methods for achieving successful pregnancy. We employed these methods, respectively, to study over 120 infertile women from June 2008 to January 2009. We divided our patients into two different age groups, 77 patients below the age of 35 (group A), and 43 of them above 35 years old (group B). ZIFT was used for 82 patients, among them 55 were belong to group A and 27 patients were belong to group B.

The results indicate 63.3% of success in patients below 35 years old, while we succeeded around 52% of pregnancy in patients above 35 years old. IVF is also used for process of conceiving in 38 patients, 22 patients below 35 years old and the rest above, respectively. The younger group shows 45.5% successful pregnancy, whereas only 37.5% positive response has achieved in patients above 35. The above data suggests that at our clinic patients who have had multiple failed IVF, or not to have patent tubes and they are older should consider ZIFT as a treatment option. Estradiol valerate was prescribed for 74 patients in groups A and B, in 62 patients who used estradiol valerate (1–2 mg) in their first and second period of cycle (days 1–7 and days 7–12) in group A, 63.6% pregnancy was achieved; whereas 58.6% positive response was found in group B. In another case in 12 patients from group A, who were prescribed estradiol valerate in their second period of cycle, we achieved 50% success. These results noted that patients who used estradiol valerate in their first and second cycles show a better results compared to the patients who received the same dose in their second cycle.

**T-091 Infertility diagnosis**

**Multiple testicular biopsies and infertility diagnosis**

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**Introduction** We were aimed at comparing results obtained from testicular biopsies with regarding histopathological patterns with due attention to the number of biopsy sites received for investigation of male infertility.

**Materials and Methods** Testicular biopsies of 6317 non-obstructive azoospermic infertile males were categorized into 6 groups: sertoli cell only syndrome (39.9%), maturational arrest (36.3%), tubular hyalinization (12.77%), hypospermagenesis (10.21%), Mixed germ cell Aplasia with Focal Spermatogenesis (MGAFS) (6.84%) and mixed germ cell aplasia without spermatogenesis (4%). Subgroup analysis included age, number of biopsy site, unilateral versus bilateral biopsies and morphologic diagnosis. MGAFS diagnosis was found in 9% of the patients underwent more than one sufficient biopsies and in 5.1% of single biopsies. The positive correlation between the presence of mixed germ cell aplasia with focal spermatogenesis and the biopsy number observed by applying chi-square test (p-value < 0.001) while there was no significant relation regarding the uni- versus bilateral biopsy.

**Conclusions** It was significant from the present study that multiple testicular biopsies will raise the possibility to detect the exact diagnosis especially MGAFS.

**T-092 Infertility diagnosis**

**Hysteroscopy, a minimally invasive method for the diagnosis and treatment of uterine infertility**

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Hysteroscopy is an endoscopic procedure which consists of introducing a telescope connected to a camera through the dilated cervical canal into the uterine cavity for the visualisation of the cervical canal, uterine cavity and tubal ostiums. It is a minimally invasive method that allows diagnosis and/or treatment of a wide variety of pathologies affecting the proper functionality of internal female genitalia (menstrual and reproductive function).

**Objectives** Our study seeks to examine both the frequency and types of endouterine pathology involved in female infertility (intrauterine adhesions, fibroids, polyps, endometritis, adenomiosis).

**Materials and Methods** The study was conducted on 105 infertile patients admitted in “Cuza Voda” Hospital, Iasi, between January 2008 and January 2010.

**Results** We investigated patients aged between 22 and 41 years (mean 31.5 years) with primary infertility: 40 cases (38.1%) and secondary infertility: 65 cases (61.9%). All patients were examined by hysteroscopy combined with laparoscopy for an infertility investigation protocol under general anesthesia. There was no recorded incident or complication. In 31 cases (29.52%) the hysteroscopic aspect was normal (other causes of infertility) and in 74 cases (70.48%) it revealed various pathologies, as follows:

- Intrauterine adhesions: 35 cases (33.3%)
- Polyps: 18 cases (17.14%)
- Endometritis: 10 cases (9.52%) – certified by anatomic-pathological examination
- Hysterophaty/atrophy of the Endometrium: 31 cases (29.52%) – discrepancy with menstrual cycle phase

There were no diagnosed intracavitary fibroids.

**Conclusions** Hysteroscopy proved to be a useful and relatively “easy to perform” maneuver in the diagnosis and treatment of endouterine pathology. It is important to note that one can identify diseases not diagnosed by ultrasound or hysterosalpingography (endometriosis and filmy adhesions).

**T-093 Infertility diagnosis**

The use of transvaginal hydrolaparoscopy in diagnosis of tubal infertility

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**Objectives** The aim of this study is the assessment of usefulness the transvaginal hydrolaparoscopy in diagnosis of tubal infertility.

**Materials and Methods** Hydrolaparoscopy (T.V.E.) was performed in 22 patients. We had previously carried out the HSG in all patients. Hydrolaparoscopy ought to be verified procedure. We performed diagnostic laparoscopy as a pilot study in 6 cases. We estimated the tubal blockage, pathological changes in Fallopian tubes and peritubal adhesions. Transvaginal hydrolaparoscopy and laparoscopy were performed under general anesthesia and HSG under local anesthesia.
Results In 2 from 22 patients we could not introduce the needle through the peritoneum of the pouch of Douglas and enter the abdominal cavity. In 2 patients we could not visualize the entire Fallopian tubes due to extensive peritubal adhesions—previously performed procedures on adnexa in anamnesis. We assessed 36 Fallopian tubes. We confirmed patency using T.V.E. in 26 cases, tubal obliteration in 8 cases and unclear image in 2 cases. In HSG we had 22 patent Fallopian tubes, 6 obliterated and 8 with unclear image. Results of both procedures T.V.E. and HSG were coherent in 18 cases of patent Fallopian tubes and 4 obliterated.

Taking into account tubal changes we detected in T.V.E.: 9 Fallopian tubes with segmental distention, 3 hydrosalpinx, 24 without any changes.

In HSG we observed 4 Fallopian tubes with segmental distention, 1 hydrosalpinx, unclear image in 8 cases, 23 with normal morphology. Results of both procedures were coherent in high percentage in cases without any morphological changes – 22 cases, only 4 with segmental distention, 1 hydrosalpinx and even 2 unclear cases from 8 observed in HSG. Peritubal adhesions were detected in 11 cases in T.V.E., 21 in HSG. The accuracy was 13. T.V.E. had higher sensitivity in assessment segmental distention and peritubal adhesions comparing to laparoscopy. The cases of tubal obliteration or patency seen in T.V.E. were confirmed also in laparoscopy. Laparoscopy turned out to be good method for verification of unclear cases.

Conclusions
1. T.V.E. comparing with laparoscopy is very useful method in assessment of tubal pathology. It is more sensitive than HSG.
2. T.V.E. is less invasive method than laparoscopy but adequate for properly chosen group of patients.
3. The final assessment of T.V.E. procedure will be possible after carrying out more studies. Our conclusions are the initial observations.

T-094 Infertility diagnosis
Resistant ovary syndrome (Savage syndrome) to gonadotropins and pregnancy

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Introduction Patients with the resistant ovary syndrome are distinguished from those with premature ovarian failure by the presence of follicles in the ovary. The resistant ovary syndrome is rare. Women with this syndrome require hormone replacement therapy and long term surveillance. With recent advances in assisted reproductive technologies, especially in in vitro oocyte maturation, distinguishing premature ovarian failure from the resistant ovary syndrome is important.

Case Report A 27-year-old null gravida woman presented with secondary amenorrhea and infertility. Her menarche occurred at 16 years of age and was followed by fairly regular menstrual cycles of 30–35 days until the age of 21, when the cycle length changed from 40–60 days. At that time, her menses became more irregular, until they eventually ceased at 25 years of age. Routine laboratory tests showed serum FSH levels of 19–29 IU/L and LH levels of 16–30 IU/L. The E2 level was then less than 25 pg/ml serum levels of prolactin, androstenedione and DHEAS were normal. The inhibin B level was 52 pg/ml. Thyroid and adrenal hormone levels were normal, and no autoantibodies against thyroid, adrenal, or ovaries were detected. Karyotype was 46XX; laparoscopy was performed for ovarian biopsy. Histological examination showed thickening of the ovarian cortex. There were prismatic follicles with few deep-lying primary and second oocytes. The ovarian stroma was thickened, and there were no cystic follicles. Administration of GnRH analogue short protocol (decaproyl 0.1 mg) and rFSH (Gonal F 75 IU), 375 IU/day for 14 days, did not influence the E2 level (17 pg/ ml), and the patient did not menstruate.

The patient was then treated for hormone replacement with 2 mg ethinylestradiol and 5 mg norgestrel per day administered sequentially for 3 months.

In a second attempt to achieve ovarian stimulation, 575 IU of HMG (Menogen®) and 2 mg ethinylestradiol per day (Ormonone®) were administered for 16 days. This treatment resulted in follicular maturation, as determined by repeated ultrasound and hormone analysis. She had a successful pregnancy with IVF and had delivered by caesarian section at term.

Conclusions We conclude from this observation that laparoscopy and ovarian biopsy are mandatory parts of the diagnostic procedures in younger women suffering from hypergonadotrophic hypergonadism. We can speculate that improvement in in vitro oocyte maturation techniques may offer hope for fertility in women with resistant ovary syndrome.

T-095 Infertility surgery
Microhysteroscopic correction of proximal tubal blockage with application of catheter stone retrieval basket

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Microhysteroscopic correction of uterine tubal junction was performed in 27 subfertile women aged 24–35 years with proximal tubal blockage. Microhysteroscopy was performed simultaneously with catheterization of interstitial segment using stone retrieval catheter.

Patency was achieved in 36 tubes out of 42 with interstitial blockage. Pregnancy occurred in 12 women (46%) within 1 year (2–9 ovulation cycles). In 3 women with unilateral blockage intrauterine pregnancy was achieved through the reconstructed tube. Ectopic pregnancy occurred in 2 cases (4.8%) on the reconstructed side.

Thus, microhysteroscopic correction of uterine tubal junction and application of functional catheter with opening basket end may be considered as a method of choice before IVF.

Background Asherman’s syndrome is a clinical entity that may present with subfertility. Outcome of management depends on severity of intrauterine adhesion and type of treatment intervention employed.

Objective Evaluation of etiologic risk factors, fertility and pregnancy outcome following treatment.

Patients and Methods A 5 years prospective observational study. Treatment employed includes transvaginal blind intrauterine adhesiolysis, insertion of inert intrauterine device or inflated Foley’s catheter balloon and oestrogen therapy. Inclusion criteria were Asherman’s syndrome as the only identifiable cause of infertility and post treatment follow up for at least a year.

Results Over a period of 5 years, 63 patients with mean age of 31.6 years and age range of 21 to 42 years were managed. Etiologic risk factors were dilatation and curettage 33 (52.4%), vacuum aspiration 13 (20.6%), myomectomy 11 (17.5%) and caesarean section 6 (9.5%) patients. Of the 63 patients, 28 conceived giving a pregnancy rate of 44.4%, 9 out of the 28 patients that conceived had miscarriage, giving a miscarriage rate of 32.1%. Highest pregnancy rate and lowest miscarriage rate was recorded in the subgroup that had vacuum aspiration as their etiologic risk factor. Late pregnancy complications encountered were preterm contractions 5 (26.3%), placenta praevia 4 (21.1%), morbidity adherent placenta 5 (26.3%) and preterm delivery in 2 (10.5%) patients. Route of delivery was per vaginam in 13 (68.4%) patients and by caesarean section in 6 (31.6%) patients. Live birth rate was 89.5% (17/19 deliveries). There was no maternal mortality recorded.

Conclusions Compared to other risk factors, uterine vacuum aspiration was associated with higher fertility rate and better pregnancy outcome.
Background Reproductive surgeries are not uncommon surgical procedures in sub-Saharan Africa where infertility cases are highest and its effect on the patient, family and society are enormous. These procedures are either done for diagnostic or therapeutic purposes to conserve, correct or improve reproductive function. Determining the pattern of reproductive surgery in a resource constrained setting like ours would help plan public enlightenment, education program on prevention, early detection and surveillance for these common pathologies mostly associated with infertility in our environment.

Objective To analyse the pattern of reproductive surgeries performed among patients that presented with infertility.

Methods A retrospective analysis of reproductive surgeries performed in infertile women between 2006 and 2009 at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Results Over a 4-years-period, a total of 235 reproductive surgeries were performed on women desirous of pregnancy. During the same period, 826 gynaecologic surgeries were done giving reproductive surgery rate of 28.5%. Reproductive organs operated on are; uterus 141 (60.0%), fallopian tube 78 (33.2%), vagina/external genitalia 9 (3.8%) and ovary in 7 (3.0%) patients. Of the 235 reproductive surgical procedures, 74 (31.5) were diagnostic procedures while the remaining 161 (68.5%) were therapeutic surgeries to conserve, correct or improve reproduction. The 74 diagnostic procedures are laparoscopy/dye hydroablation in 68 (91.9%) patients and biopsy of adenomyosis in 6 (8.1%). The 161 therapeutic procedures are intrauterine adhesiolsis 68 (42.3%), myomectomy 67 (41.6%), tuboplasty 10 (6.2%), feminizing genitoaplay for female intersex 5 (3.1%), ovarian cystectomy 4 (2.5%), wedge excision / ovarian drilling for polycystic ovarian syndrome 3 (1.3%) and 2 (1.2%) patients with excision of transverse vaginal septum.

Conclusion Diagnostic laparoscopy/dye hydroablation, intrauterine adhesiolsis and myomectomy are the three most common types of reproductive surgery performed and they account for about 85% of all reproductive surgeries done within the study period.


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Objective
1. To study robotic setup time.
2. To determine the optimal scope position in each uterine tube dissection.
3. To evaluate postoperative surgical wound.

Design Descriptive study.

Setting Faculty of Veterinary Medicine, Khon Kaen university, Thailand.

Patients Female pigs, 3 months of age, were assigned for this robot-assisted tubal surgery.

Interventions A novel laparoscope hold- ing robot, which received very encouraging an IFIA gold medal award in 2008, was tested for the feasibility and safety. Ten experiments of robot-assisted tubal surgery were performed on female pigs. The duration of machine setup was recorded. Then, the optimal angles of each uterine tube were identified in horizontal angle (X) (the angle between laparoscope axis and mid-sagittal plane), and vertical angle (Y) (the angle between laparoscope axis and a line between Xyphoid and umbilicus). The optimal depth of laparoscope position was recorded. Finally, after laparoscope-cannula removal, the incised wounds were explored to identify the unexpected trauma causing by robot arm movement.

Results The median of operating time was 44.5 min (range 11–79 min). The median duration of machine setup was 3 min (range 2–11 min). The median optimal angle in operation of right uterine tube was 14 degree (range 3–30 degree) in horizontal plane (X) and 28 degree (range 24–43 degree) in vertical plane (Y). The median optimal angle in operation of left uterine tube was −10 degree (range −4 to−25 degree) in horizontal plane (X) and 29 degree (range 24–51 degree) in vertical plane (Y). The median optimal depth of laparoscope position in right uterine tube opera- tion was 9.5 cm (range 7.5–12.5 cm) and left was 9.5 cm (range 6–12.5 cm). The experiments were completed successfully. No complications occurred. The laparoscope-cannula incised wound were not showed abrasion or tear causing by robot arm movement.

Conclusions Our novel laparoscopic holding robot was easy to setup and work effectively on porcine tubal operation. We hope to implement this convenience robotic surgery in wide variety of gynaecologic surgery from robot-assisted laparoscopic tubal sterilization to robot-assisted laparoscopic tubal reanasto- mosis.

T-099 Infertility surgery Successful treatment of pregnancy in lower uterine segment with direct injection of methotrexate

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Aims Ectopic implantation in the lower uterine segment including caesarean scar and uterine cervix is the rarest form of ectopic pregnancy. These are considered even more aggressive than placenta previa because of its earlier invasion of the myometrium. Such cases are at very high risk for uterine rupture with maternal life-threatening complications; therefore, hysterectomy is frequently chosen. Here we present the successful conservative management and clinical course of a pregnancy in the lower uterine segment treated by local injection of methotrexate (MTX).

Methods We undertook a retrospective analysis of pregnancy in the lower uterine segment, including 4 patients with caesarean scar pregnancies and 6 with cervical pregnancies, treated by local injection of MTX at our hospital, between April 2008 and March 2009. The locus of ectopic gestational sac was evaluated by transvaginal ultrasonography, followed by ultrason-guided injection of 50 mg of MTX into the gestational sac and surrounding uterine myometrium. We analyzed the clinical symptoms, complications, and weekly serum hCG measurements until these values return to normal.

Results The mean gestational age at local injection was 8.0 ± 1.9 weeks. Serum hCG levels at treatment varied from 2538.6 to 87517.2 mIU/mL. Serum hCG was sustained until one day after injection and then started to decline. Treatment was achieved by a single injection except in two cervical pregnancies that required additional local MTX injection on postoperative day 5 or 6 because of sustained serum hCG. None of the patients was complicated by massive haemorrhage. In 9 of these 10 women, serum hCG concentration spontaneously returned to normal and no additional intervention was required, whereas one case of caesarean scar preg- nancy received systemic methotrexate because her serum hCG remained elevated 43 days after MTX injection.

Conclusions Ectopic pregnancy in the lower uterine segment is a burden for not only the obstetrician but also for women who wish to preserve fertility. Transvaginal injec- tion of methotrexate is a simple and useful method for conservative management of ec- topic pregnancy in the lower uterine seg- ment. Weekly serum hCG measurement is useful to monitor the outcome of local MTX injection and ensure successful treatment.
T-100 Male infertility
Immunological factors affecting male fertility – state of the art
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Aims Present epidemiological studies acknowledge that among the infertile population at least 5–10% of couples may present circulating or locally secreted antisperm antibodies (AsA) that may impair human infertility. AsA antibodies may block transport of spermatozoa (sperm agglutination and/or immobilization) including cervical mucus ‘shaking phenomenon’, and may inhibit sperm-oocyte interaction or early embryo development at first two weeks after fertilization. New modes of action therefore should be recommended taking into account natural pro-creation or techniques of assisted reproduction (ART).

Methods Diagnostic tests should, first of all, assure presence of live spermatozoa (mixed agglutination reaction (MAR) or immunobead test (IBT) should be first in a line as it is recommended by the World Health Organization) and studies of free or bound-to-spermatozoa antibodies shall be advised (indirect or direct assays). Cut off values for ASA-bound-to-sperm are critical for further therapeutic proposals.

Results Cut-off at the range of 25% bound sperm can be considered as patognomic for natural intercourse, 50% for more aggressive approach (IU intrauterine insemination) while 75% of bound sperm (AsA) would require assistant reproduction (IVF, ICSI). At this level when two classes of AsA antibodies are encountered (IgG, IgA) then combination of ART with immunosuppressive regime (pharmacological schemes of steroid application) or ART with sperm fractionation would be required. European and American schemes differ at degree of invasiveness when AsA are encountered.

Conclusions Final outcome will be still uncertain when more immunological disturbances will be met on either or both sides (partners) – as cytokines, signs of autoagression etc. Some clinical variants will be discussed.

Wednesday, September 15
W-001 Male infertility
Fertility score elaboration after examination of sperm morphology and additionally analyses of motile sperm
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Couple infertility is circumscribed to the modern concept of reproductive medicine. European statistics reveal a birth decrease due to the living standard increase, socioeconomic changes and infertility in adult population at the reproductive age. Infertility prevalence in Europe is an argument topic for sociologists and statisticians. As far as this aspect is concerned, it has been shown that the percentage of pregnancies registered in one year, in the case of a couple belonging to the optimal age interval decreased from 86% to 52%. Compared to 1970, a strong fertility decrease is noticed at the level of the entire European Community. Couple infertility causes imply female infertility, male cause infertility, both partner’s infertility, and infertility of unknown cause. In the case of male infertility, one may notice an increased percentage compared to the one registered 10 years ago. The poorer quality of spermatozoids is regarded as the main responsible factor. Spermogram parameters have changed, as there are decreased reference values in the case of the sperm volume, the total spermatozoid number or concentration per milliliter. According to the new reference values of the World Health Organization, the morphological criterion has a new value, a more important one, since minimum 4% morphologically normal spermatozoids are identified. The abnormal spermatozoid percentage is thus established too which are classified as amorphic. Each of these abnormal forms is not able to fertilize the oocyte. Moreover, the genetic information, the DNA may be fragmented, which may lead to the occurrence of aneuploidy. The spermatozoid morphological study criteria are included in the Tygerberg criteria widely used in the evaluation of the spermatozoid fertilization capacity. But it is not limited to this aspect. It also represents a diagnostic criterion for a complete spermogram, by means of which one may establish whether spermatozooids are apt to fecundate the ovocyte or they may be used in intrauterine insemination. The costs of such a procedure are substantially high. Therefore, the morphological criterion receives even more importance in the accurate and complete elaboration of a male infertility diagnosis. Elaboration of fertility score will have practical applicability in increased success rate of intrauterine insemination and possibilities of diagnosis and subsequent elaboration of therapeutic behavior in the case of male infertility.

W-002 Male infertility
Does magnetic field affect the testicular ultrastructure?
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Objectives To investigate the possible testicular ultrastructure changes following the exposure of rats to the magnetic field of high voltage electric transmission lines.

10 adult male rats were placed in the vicinity of a high voltage electric transmission line for 56 days and a control group of 5 rats were placed away from the magnetic field. Tests were dissected and sections were examined under both light and transmission electron microscopes. Light microscopy showed no gross morphological changes of the seminiferous tubules and the spermatids. However, electron microscopy showed polymorphic mitochondria with irregular cristae in the Sertoli cells and much lipid droplets in the cytoplasm of the germ cells of the testes of rats placed in the magnetic field. Such changes might affect the spermatogenesis in case of prolonged exposure to a magnetic field of high strength.

W-003 Male infertility
The evaluation of male infertility in infertility department of Sarem Women’s Hospital: A 10 year survey
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Background Infertility is a problem affecting an estimated 10–15% of all couples. Both man and woman are involved in this problem. It is estimated that male factors are causing 40–50% of all infertilities. To evaluation the prevalence and main causes of male infertility, a long-term survey was conducted over 10 years (1997–2007) in patients referred to Infertility Department of Sarem Women’s hospital.

Method In a retrospective study, the medical records of 5132 infertile couples referred to Infertility Department of Sarem Women’s Hospital were evaluated and main causes of male infertility were determined.

Results This study shows that, 2979 among 5132 infertile couples had pure male-related infertility (58.0%) and 2006 couples had both male and female-related infertility (39.1%). The main causes of male infertility were teratospermia (40.9%), asthenoterato-spermia (23.9%), oligo-asthenoteratospermia (12.0%) and azoospermia (11.2%). Less cause of male infertility was oloigospermia (0.1%). 1385 males had varicocele (27%). 80% of oligo/azoospermia cases had FSH level less than 10 IU/L and 82% of them had LH level less than 15 IU/L.

Conclusion The high prevalence (97.1%) of male infertility was seen in this study, comparing to the prevalence reported of male-related infertility in other studies ranging from 40–50%. Many factors such as environmental pollutions (air and water), chemical poisoning and side effects of chemical wars, food additives, smoking and drugs abuse, sedentary lifestyle and obesity may affect the quality and quantity of sperm and they may increase chance of infertility. As was seen in this study, the most causes of male infertility were related to abnormal morphology and dysfunction of sperms (teratospermia and ashenotateratospermia) whereas the reduced amounts of sperms (oligo/azoospermia) are the less common cause. These evidences emphasize the main roles of
ED affect more than 150 million males throughout the word. Saffron is an old plant that is used as a herb, as appetizer, treat about mentioned diseases and even aphrodisiac. However, its effect on ED has not evaluated so far. The purpose of this prospective descriptive study is to evaluate the effect of saffron on ED.

This prospective descriptive study conducted to evaluate the effect of saffron on 20 ED male patients. Treatment period was ten days. During this period patients received a yellow tablet containing 200 µg saffron each morning, expect last day (Day 10) who scheduled to have rigiscan, and take 2 tablets.

In this study we evaluated and confirmed its effect on ED by 2 means: NPT test and IIEF-15.

All 20 cases came after 10 days for second NPT and second IIEF-15. There was a statistically significant difference in tip rigidity (p < 0.05) and tip tumsence (p < 0.05) as well as base rigidity (p < 0.05) and base tumsence (p < 0.05).

Among many herbs, saffron is known as an aphrodisiac agent from many centuries ago. But its validity efficacy was remained.

Our study showed the potential effect of saffron on ED by two methods: NPN and IIEF-15 tests. Currently, the first line of ED therapy with any reasons is phosphor diesterase-5-inhibitors but they are costly and have many side effects. Saffron tablets have no side effect, it is cheap and it is growing especially in our environment. Saffron tablets have no side effect, it is known.

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In the present study, it is shown that saffron has statistical significant effect on men with erectile dysfunction by NPT and IIEF-15 tests. It is cheaper than phosphor diesterase 5-inhibitors and with compare to them has no side effect with 200 µg/day. It is possible to replace this yellow herbal tablet to phosphodiesterase 5-inhibitors by doing more research in more patients.

W-005 Male infertility
The effects of long-term obstruction on the efferent ductules and their contents: 2 case reports and comparison with a normal caput epididymis

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Introduction Vasectomy and any long-term obstruction of the upper efferent ducts of the testes results in profound changes to the histology of the ductal system distal to the obstructive lesion in particular to the caput epididymis.

Materials and Methods In this presentation, three cases are described. The first case describes the caput and the cauda of epididymis taken at autopsy from a sudden death victim showing histology of the caput and the cauda in an individual with a normal efferent duct system. The second patient is a man with an 18-year-old vasectomy who underwent an epididymectomy. The third patient is a man with an upper efferent epididymal obstructive lesion of unknown duration who underwent an epididymal biopsy during a scrotal exploration.

Results The caput epididymis is empty of sperm in the sudden death victim. This section of the caput epididymis also shows the complex epithelium of the caput. The cauda epididymis is packed with sperm confirming this part of the epididymis as a major storage area for sperm. However, in the man with the long-standing vasectomy, large numbers of sperm are now present in the caput. A high power view of this area also shows major changes to the epithelium. The principle cells are the halo cells. Very few clear cells remain. The stereo-cilia have also almost completely disappeared. These changes are repeated in the man with the idiopathic upper epididymal obstruction. The contents of the epididymal caput consist of many spermatozoa much contaminated by the presence white blood cells, histiocytes, macrophages and germinal cells suggesting the presence of large amounts of superoxide anions.

Conclusion Obstruction of the epididymal ducts and the vas deferens results in marked changes to the epididymal epithelium.

W-006 Male infertility
High prevalence of AZFb microdeletion in Iranian patients with idiopathic non-obstructive azoosperma

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Evaluation of saffron on male erectile dysfunction initial report
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Currently, the first line of ED therapy with any reasons is phosphor diesterase-5-inhibitors but they are costly and have many side effects. Saffron tablets have no side effect, it is cheaper than phosphor diesterase 5-inhibitors and with compare to them has no side effect with 200 µg/day. It is possible to replace this yellow herbal tablet to phosphodiesterase 5-inhibitors by doing more research in more patients.

W-007 Male infertility
The effect of cigarette smoking on semen quality of infertile men
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Objective To evaluate the effects of cigarette smoking on semen quality of infertile men.

Methods 214 infertile men who had been smoking cigarette and 130 infertile non-smokers’ men participated in this study. Seminal volume, sperm concentration, motility, viability, and morphology were examined.
Results The quality of spermatozoa obtained from smokers were much lower than non-smokers (p < 0.01). The sperm concentration, viability and forward progression were negatively correlated with cigarette smoking (p < 0.01).

Conclusions Smoking does affect the semen quality of infertile men.

W-008 Male infertility
Management of azoospermia in Togo: Proposition of a predictive sperm retrieval index (SRI)

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Introduction Advances in ART including ICSI, then getting pregnant with testicular sperm have change azoospermia treatment. Thus arose various methods of exploration and collection of sperm more or less traumatic, invasive and risky. From observations of our practice we are using a simple predictive Sperm Retrieval Index for obtaining sperm (called the Index 120), which guides our care in azoospermia.

 Patients and Methods This is a study both retrospective and prospective. Between 1998 and 2008 we analyzed clinical and biological (including FSH level) data of azoospermia patients, treated at the Clinique Biais Lomé.

For these patients, azoospermia was confirmed by at least 2 semen analysis. Sperm were collected by Microsurgical Epididymal Sperm Aspiration (MESA) or Testicular sperm Extraction (TESE).

Finding from these results, a correlation between testicular volume and, a low level of FSH with good results of MESA let us to imagine a predictive relation: (FSH X 10) – testicular volume = SRI. When SRI is below than 120, we have all the chances to find sperm by MESA or by TESE. After the establishment of this Index any surgical sperm retrieval is guided by it. We present the results of our work and the arguments for validation of this Sperm Retrieval Index “Index 120”.

W-009 Male infertility
Etio-pathological analysis of 200 cases of azoospermia seen in a private infertility center

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Introduction The number of couples affected by infertility is currently estimated to be 15% of all couples attempting to have children. The difficulties are attributable to significant male factor alone in 30% of couples and to a condition of combined male and female factors in an additional 20%, the rest being due to female factor alone or unexplained infertility.

Objectives To arrive at an etio-pathological diagnosis of 200 cases of azoospermia attending a private infertility center at Mumbai, in the last decade.

Materials and Methods A prospective study of 200 patients with azoospermia by evaluation of their clinical presentation, hormonal status, and testicular biopsy.

Results and Discussion
1. Testicular volume and basal plasma FSH levels were found to be the most useful parameters in evaluation.
2. Hypogonadism with azoospermia was seen in several patients requiring institution of androgen therapy. We also came across Fertile Eunuch Syndrome, bitropic Gonadotropin Deficiency as well as karyotype abnormalities.
3. Prolactin levels were useful only in those males (1 in our study) who presented with sexual dysfunction.
4. Semen volume, fructose, pH was invaluable in confirmation of congenital absence of vas deferens and seminal vesicles and in ejaculatory duct obstruction. The above can be confirmed by transrectal ultrasound.
5. Testicular Biopsy is useful in a limited number of cases.

Detection of AZF on the long arm of the Y chromosome although desirable is not available in our city and hence could not be studied. Based on our study a simple flow chart for the etio-pathological diagnosis of azoospermia has been devised.

Conclusion Azoospermia is not a dead end in the management of male infertility. In our patients we found that therapy could be offered in the form of NSAIDS, antibiotics, clomiphene citrate, androgen therapy, gona- dotrophin therapy, micro-surgical intervention and therapeutic donor insemination, etc. ICSI can be advised to patients who can afford it. Adoption advice should not be overlooked.

W-010 Male infertility
History of cryptorchidism and ejaculate volume as simple predictive parameters for the presence of testicular sperm

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Introduction Testicular volume, hormones and growth factors are used for prediction of the chance to find motile testicular sperm in azoospermic men. In this study, the possible predictive value of very simple parameters such as systematic history, clinical examination, and determination of ejaculate volume was evaluated.

Materials and Methods 262 consecutive non-varicoceleted men with azoospermia/asterepermia were evaluated by systematic history, clinical examination, ultrasound, semen analysis, karyotyping and examination for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations and Y microdeletions.

Results In 76 (29%) probable genetic causes were detected. For men with at least one CFTR mutation, motile sperm could be detected in 100% of 13 men with Congenital Bilateral Absence of Vasa Deferentia (CBAVD) but only in 44% of 18 with present Vasa Deferentia (VD). Ejaculate volumes were significantly lower (2.5 mL against 3.6 mL) in 81 men with motile testicular sperm detected compared to 111 men without detectable motile sperm (p < 0.001, Student’s t-test). Furthermore, an inhomogeneous histological pattern with maturation of sperm in little islands isolated in tissue showing Sertoli cell only-pattern seems characteristic for men with a history of cryptorchidism (negative predictive value: 95%).

Conclusions In addition to FSH, testicular volume, and other endocrine factors, it is important to remember that very simple factors such as ejaculate volume and presence or absence of VD in men with CFTR mutations might be used as prognostic factors according to the chance of finding motile testicular sperm. Evidence for a strong association between a history of cryptorchidism and an inhomogeneous histological pattern with maturation of sperm in little islands in tissue showing only Sertoli cells might indicate that multiple TEsticular Sperm Extractions (TESEs) should be considered particularly in men with a history of cryptorchidism.

W-011 Male infertility
Hyaluronic acid binding by human sperm in patients with varicocele

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Varicocele has been associated with decreased semen quality. However the conventional semen analysis does not give information on quality of sperm membrane. Recently, a very simple test, the sperm-hyaluronan-binding assay has been developed as a commercial diagnostic kit for assessing sperm maturity and function. The purpose of this study was to determine both sperm-hyaluronan-binding assay (HBA) and hypoosmotic swelling test (HOST) as markers of the functioning of sperm membrane, to establish the possible correlation with varicocele and with classic seminal parameters, such as sperm concentration, motility and morphology. Patients with varicocele grade II and grade III and normozoospermic men without varicocele
W-012 Male infertility
Human sperm DNA integrity versus other standard semen parameters

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DNA integrity in human sperm seems to play an important role in the outcome of assisted reproductive techniques ART, due to the effect on fertilization, embryo quality and pregnancy rates. The efficacy of regular semen analysis is questionable when the DNA integrity is increased.

The relationship between conventional sperm parameters and DNA fragmentation status is still unclear. The objective of our study was to examine the correlation between sperm parameters, such as concentration, motility and forward progression, and the level of DNA integrity.

The analyses were done with the indirect assay (Sperm Chromatin Structure Assay; SCSSA) and the genetic integrity was identified by DFI (DNA Fragmentation Index) representing the DNA strand breaks. In our study we’ve only included patients undergoing fertility treatment. We have found a significant negative correlation between genetic integrity and sperm concentration, motility and rapid motility. Patients showing a high DFI reveal reduced sperm count and impaired motility in all three DFI groups (< 15%, > 15% and > 25%).

We advise that the assessment of the sperm DNA damage should be a part of the initial semen analysis for fertility patients. Further data analysis is needed to investigate the predictive value of the DNA fragmentation index in ART cycles.

W-013 Male infertility
CAG polymorphism and mutations of the androgen receptor genes in fertile and infertile agriculture men

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Introduction: The beginning of the spermatogenesis is linked to cellular responses to androgens. The hormonal action of 2 important androgens (testosterone and 5α-dihydrotestosterone) is mediated by the androgen receptor (AR). The aim of this study was to molecularly characterize the mutations and the CAG polymorphisms in a population of infertile men who live in a rural region, with the diagnosis of idiopathic infertility.

Materials and Methods: In this prospective study, we compared 45 rural men with idiopathic infertility with 45 rural men who had a previous child in the last year. In the group of infertile men, all women had a child from a previous relationship. DNA was obtained from the lymphocytes’ blood and from cells of the oral mucosa through smear provided in a commercial kit (Wizard Genomic Purification) used to evaluate the AR.

Results: Differences were detected in the semen analysis in the group of infertile men compared to normal semen donors: sperm concentration (mean ± SD) (30.1 ± 36.5 vs 57.1 ± 23.2; p = 0.03), sperm motility (40.93 ± 20.7 vs 52.3 ± 14.5; p = 0.03), sperm morphology according to the WHO (10.7 ± 6.55 vs 36.5 ± 4.2; p = 0.03) and the specific criteria (0.3 ± 1.1 vs 3.8 ± 2.7; p = 0.03). These results were consistent with the findings of our previous study.

Conclusions: We observed a significant correlation between the CAG polymorphisms and sperm morphology according to the WHO and the specific criteria. These findings suggest that the CAG polymorphisms may play a role in the infertility of rural men.
W-015 Male infertility
Leukocytospermia and its correla-
tion with sperm parameters in infertil-
e male subjects
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Republic of Iran

Introduction Peroxidase positive leuko-
cytes are the main source of reactive oxygen
species (ROS) in the seminal fluid. They are
derived from abnormal sperm, white blood
cells or both. Oxidative stress will cause lipid
peroxidization, sperm motility defects and de-
creases in sperm fertilizing capacity. The aim
of this study was to determine sperm quality
(Sperm count, motility and morphology) in infertil-
e men with or without leukocytos-
permia and measure both malondialdehyde
(MDA) production in semen and its total an-
tioxidant capacity (TAC).

Materials and Methods In this study,
110 male subjects referring to Kashan Infer-
tility Center during 2007–2008 were divided
into two groups of leukocytospermic and non-
leukocytospermic, based on routine semen
analyses. Regarding the World health Organiza-
tion criteria, semen samples from 45 patients
were classified as normal and 30 were leuko-
cytopemic and 35 leukocytospermic; although there
were < 0.25 × 10⁶ leukocytes in the semen of the lat-
ter group. Sperm motility (a+b) had a signifi-
cant difference in the leukocytospermic indi-
viduals compared to non-leukocytospermic
or healthy subjects (p < 0.05 and p < 0.001,
respectively). MDA levels in samples from
leukocytospermic group (178 ± 18.48) were
higher than those of the non-leukocytos-
permic group (2.7 ± 1.73) and control group
(0.4 ± 0.14); the difference was also signifi-
cant between the controls and the leukocy-
tospermic subjects and between non-leuko-
cytopemic and leukocytospermic individu-
als (p < 0.001 and p < 0.0001, respectively).
Additionally, TAC levels in the leukocy-
tospermic group were significantly lower
than the other two groups (p < 0.0001).

Conclusions Presence of leukocytes in
 seminal plasma was associated with a signifi-
cantly increased ROS-producing. ROS and
MDA with oxidation of antioxidant compo-
ents are the result of TAC level reducing in
 seminal plasma. Decrease of TAC and MDA
levels were affect sperm motility. Sperm mo-
tility has principal effect on fertilization rate,
and leukocytospermia may positively influ-
ence on fertility in infertile couples.

W-016 Male infertility
Spermatoc vein embolization im-
proves semen quality in infertile
men
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Introduction Varicocele is considered a
major cause of male infertility, with an over-
all prevalence rate of 40% in men being
treated for primary infertility and of 80% in
men with secondary infertility. Varicocele
causes a progressive deterioration of seminal
quality and its repair seems to improve sper-
matogenesis. However this assumption re-
mains controversial. The aim of this study
was to assess the effect of spermatic vein em-
bolization before ICSI in semen quality in a
population of infertile men.

Materials and Methods A retrospective
cohort of 34 infertile men of mean (± SD)
age 33.64 (± 4.41) years who underwent
spermatic vein embolization with coils be-
tween February 2008 and September 2009 in
University Hospitals of Coimbra were evalu-
ated for semen quality according to WHO
criteria. All patients underwent a complete
history, physical examination, hormone pro-
file and genetic testing if needed (karyotype
and Y microdeletions). Embolization with
coils of the internal spermatic vein was per-
formed after venography. Semen analysis
was performed before embolization and re-
peated 4–6 months after the procedure. A
positive effect of the procedure was consid-
ered when seminal parameters rose from
abnormal to normal values according to
WHO criteria.

Results Significant improvement was ob-
served in sperm concentration, motility and
morphology after spermatic vein emboliza-
tion. After this procedure, sperm concentra-
tion increased from 27.5 ± 4.0 × 10⁶/ml to
36.4 ± 7.4 × 10⁶/ml (p < 0.05); sperm rapid
progressive motility rose from 0.8% ± 0.5 to
4.8% ± 1.8 (p < 0.05), and sperm normal
morphology rose from 5.2% ± 1.0 to 9.4% ±
1.3 (p < 0.05). Besides, normal sperm con-
centration was seen in 52% of the individuals
before treatment and in 56% after treatment.
Similar results were observed for progressive
motility (increase from 36% to 52% of men
and normal morphology that was observed in
4% of men before treatment and in 16 after
treatment. Although we observed that the
procedure induced a significant improve-
ment in one seminal parameter for 38% of the
individuals, in two seminal parameters for
8% and in all of the semen parameters in 4% of
patients. Furthermore, preliminary data
suggest that, after treatment, there is a de-
crease in sperm chromatin damage.

Conclusions Spermatic vein embolization
improves sperm quality, namely sperm con-
centration, progressive motility and mor-
phology in an infertile population. Further-
more, the treatment seems to improve sperm
chromatin integrity.

W-017 Male infertility
Normal live birth after TESE and
ICSI in variant primary ciliary dys-
kinesia with completely immotile
sperm and structurally abnormal
axonomes
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Complete sperm immotility suggests struc-
tural sperm anomalies, especially primary
ciliary dyskinesia (PCD), a genetically het-
erogeneous syndrome affecting in ~1–20,000
men. PCD is often associated with recurrent
respiratory infections, infertility, and when
situs inversus is present, termed Kartagener
syndrome. These “ciliopathies” are linked to
defective axonemal structure/function and
generic associations are recognized. Several
reports of live births following ICSI in PCD
have appeared.

We report a 40 year old infertile man with
variant PCD featuring mild respiratory cilia
dysfunction, and 100% immotile and struc-
turally abnormal sperm. His reproductive
history was normal but respiratory history
included severe neonatal chest infection,
mild asthma, recurrent chest infections and
repeated nasal surgeries. Physical examina-
tion, serum FSH, LH and testosterone levels,
karyotype and Yq structure were normal. Sperm
density was 10 millions/ml, 100% immotility,
and vitality 16% and many sperm having short/absent
tails.

Transmission electron microscopy of sperm
showed severely disrupted axonemes and outer
dense fibre formation. By contrast fi-
brous sheath structures were relatively nor-
mal. Many sperm also showed abnormal cent-
riole structure at the base of the sperm head
and many were decapitated. A nasal biopsy
revealed a ciliary beat frequency below nor-
mal, but greater than in classic PCD, and
most did not fully flex and had a dys-syn-
chronous beat.

To optimize the prospects of viable sperm
with intact DNA and normal centriole struc-
ture for ICSI, bilateral TESE was performed
but all sperm remained immotile after 3 hrs
incubation. The most morphologically nor-
mal sperm, showing tail fluidity and re-
mainit intact during micromanipulation,
were selected; 12:18 eggs fertilized and one
blasto cyst was transferred and 2 vitrified on
Day 5. A normal pregnancy resulted with de-
livery of a healthy 3840 g, female infant at 38
weeks gestation.

Non classical PCD may present with struc-
turally abnormal, completely immotile sperm
with reduced vitality, perhaps reflecting pro-

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suggested to be an excellent dietary supplement to help maintain male fertility that can be compromised due to OS.

W-019 Male infertility

Effect on the damage of testicular germ cells and its Bcl-2/bax expression induced by P-nonylphenol

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To explore the effects of damage of testicular germ cells induced by a p-nonylphenol injection, wild-type C57 mice were injected with p-nonylphenol (10%, v/v) for 7 or 14 days. The damage and apoptosis of testicular germ cells were evaluated by using methods of hematoxylin eosin (HE) staining, DNA fragmentation test, and Western-blot. The results indicated that there was no morphological change in the epithelial cells of testicular tubules after 7-day p-nonylphenol injection, but more apoptotic germ cells were exhibited in these p-nonylphenol-injected mice than that in the wild-type mice. Furthermore, the mice subjected to a 7-day or 14-day injection of p-nonylphenol displayed a down-regulation of Bcl-2 and an up-regulation of Bax in the testis. These evidences suggested that the apoptotic regulation may be involved in the damage of testicular germ cells induced by p-nonylphenol injection, and Bcl-2/bax could contribute to regulating the apoptosis of testicular germ cells.

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W-020 Male infertility

Apoptosis and proliferation study of GnRH potential in inhibition of side effect of drugs used in chemotherapy: An ultrastructural and immunohistochemical study

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Aims Male factors, mainly spermatogenesis disorder, are responsible for 20–30% of infertility occurs in different societies. One of the known causes of spermatogenesis disorder is chemotherapy in patients with cancer. The side effect of chemotherapeutic agents may last from 10 years up to the end of the life. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist as a suppressor of spermatogenic proliferation, on spermatogenic defect produced by anticancer drug (thiopeta).

Methods In the present study 30 adult male mice aging 6–8 weeks were used. The mice were divided into 3 equal groups as: control, thiopeta (T group) and thiopeta + cetrorelix, a GnRH antagonist (T + C group). Thiopeta was injected as ip for 5 days at 2.5 mg/kg doses. In T + C group cetrorelix injection was started one week before thiopeta treatment and continued for 3 more weeks. Since spermatogenic cycle in mice is 35 days, mice in all groups were sacrificed 35 days after thiopeta injection. Half of testicular specimens were fixed in formaddehyde for LM studies (Ki-67) and others were fixed in 2% glutaraldehyde and prepared for EM studies. The thin sections were studied with LEI 906 TEM.

Results Electron microscopic study showed a lot of apoptotic cells with apoptotic bodies in spermatogony and leidyc cells and pre-apoptotic features in sertoli cells. LM study with Ki67 Kit showed that mean of prolifera- tion in control group was 9.40 ± 0.23, but in T group was 4.85 ± 0.15 and in T + C group was 9.40 ± 0.23. Statistical analysis of data show significance difference between control and T group (p < 0.05) but not between control and T + C group (p < 0.05).

Conclusion According to the result it is concluded that GnRH antagonist administration before cancer treatment could prevent the side effect of anticancer drugs.
tion and motility. The catalase activity were significantly (p < 0.05) higher in the sperm of rats consuming fermented rooibos, “green” rooibos with green tea supplement, and green tea supplement. While the concentration of superoxide dismutase in the sperm of rats supplemented with fermented rooibos, “green” rooibos, and green tea was higher (p < 0.05) compared to control. The glutathione levels of rats fed with fermented rooibos and “green” rooibos were also significantly (p < 0.05) higher. None of the tea treatments showed an effect on lipid peroxidation and reactive oxygen species level, although rooibos fermented and “green” rooibos showed a tendency to lower the levels of these 2 biomarkers when compared with the control group. Our study demonstrates that both the rooibos extracts could offer a measure of protection against induced oxidative damage by increasing the antioxidative defence mechanisms which could improve the sperm quality and function.

W-022 Male infertility
Comparison of laparoscopic high ligation and microscopic low ligation of the spermatic vein in the patients with varicocele
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Purpose We retrospectively reviewed the efficacy of surgical treatment for the patients with varicocele.

Patients and Methods Reviewed patients with grade II–III varicocele were 83 who underwent either laparoscopic high ligation (LHL, n = 57) or microscopic low ligation (MLL, n = 26) of the spermatic vein. 63 of 83 had chief complaint of male infertility. The other 20 underwent surgery for symptomatic varicocele (scrotal swelling, pain, discomfort). Patients’ age was 30 ± 8.95 years. Preoperative semen volume was achieved except for semen volume was achieved (p = 0.026). Postoperative time to start of oral food intake was shorter in MLL than in LHL (18.2 ± 3.2 hours vs 26.0 ± 2.2 hours; p < 0.001). Requirement of analgesics after surgery was less in MLL than in LHL (0.35 ± 0.19 days vs 0.81 ± 0.13 days; p = 0.0194).

Conclusion Both procedure of LHL and MLL showed a comparable improvement of semen parameters and varicocele-related symptoms. However, postoperative recovery after MLL was significantly faster than LHL with no major complications, although MLL needed more operation time.

W-023 Male infertility
Cell type related apoptosis in seminiferous germ line cells induced by chemotherapeutic agents
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Aims Infertility problem affect more than 15% of young couples in different societies. In 20–30% of cases the problem is due to male factors, mainly spermatogenesis disorder. One of the known causes of spermatogenesis disorder is chemotherapy in patients with cancer. This side effect may last from ten years up to the end of the life. Since anti cancer drugs are mainly insert their effect to male factors, complete lack of assembly of complex I, IV, V. This assembly defect occurs despite a normal and V. This assembly defect occurs despite a complete lack of assembly of complex I, IV, V. This assembly defect occurs despite a complete lack of assembly of complex I, IV, V.

Methods In the present study 60 adult male animals aged 6–8 weeks were used. The mice were divided into 3 groups: busulfan, cyclophosphamide and thiopeta, half of the mice in each group were used as control, receiving the solvent of the drug. Busulfan was dissolved in DMSO and injected as a single dose of 40 mg/kg. Cyclophosphamide was dissolved in saline and injected as 0.8 mg/day for 15 days. Thiopeta was injected for 5 days as 2.5 mg/kg. Since spermatogenesis cycle is 35 days in mice, mice in all groups were sacrificed 35 days after injection and testicular specimen were fixed in 10% formalin and paraffin embedded. Sections were stained with TUNEL technique for apoptosis detection. For electron microscopy, the specimens were fixed in 2% glutaraldehyde, embedded in araldite and thin sections were studied with LEO 905 TEM.

Results The results from histochemoical study showed that TUNEL positive cells with a yellowish brown color were mainly located among spermatogenic cells but rarely among the spermatocytes. There were 4 fold of increase in the number of TUNEL positive cells, in all experimental groups, in comparison to control group. Transmission electron microscopy were in support of histochemoical findings and revealed that apoptotic cells could easily been distinguished from normal and necrotic cells by having condensed nuclei and its separation from neighboring cells. Heterochromatic nuclei were some times had a crescent like appearance and altered mitochondria, characteristics of apoptotic cells.

Conclusion The results of the present study indicate that anticancer drugs induce apoptosis mainly in spermatogonial cells but rarely in spermatocytes.

W-024 Male infertility
The 4977 bp mitochondrial DNA deletion and tRNALeu(UUR) transition in asthenozoospermic men
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Introduction Since spermatozoa movement requires great amount of energy. Defects in respiratory function are assumed to cause a decline in motility and, consequently, decrease of fertility. The 4977-bp deletion (8469–13347) occurs between 2 13-bp direct repeats and results in mitochondrial DNA molecules (mtDNA 4977) that lack all or part of a 12 gene cluster. The A3243G transition is the most extensively investigated tRNA gene mutation produces a severe combined respiratory chain defect with almost complete lack of assembly of complex I, IV, and V. This assembly defect occurs despite a modest reduction in the overall rate of mitochondrial protein synthesis.

Materials and Methods We collected 240 semen samples from 240 infertile men with asthenozoospermia (a < 25% or a b < 50% of motility) in 1999. The semen samples obtained from 46 healthy individuals were used as control. The A3243G transition was studied by poly-
merase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis with BsuRI. Blood samples obtained from patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome) were used as control of restriction endonuclease activity.

**Results** The 4977 bp deletion was identified in 96% of analyzed samples and also in 87% control group blood samples. 13 bp direct repeats located at flanking region (8470–8482 and 13447–13459 respectively) may cause PCR slippage and artificial fragment amplification. The A3243G transition was excluded in all extremely variable (sperm motility [a+b] 3%–38%) semen samples.

**Conclusions** The results of our study correspond to the current data confirmed that the coxPCR does not allow detecting mtDNA deletion 4977 accurately. Potential use of this mtDNA deletion as a diagnostic test requires more reliable methods. It seems that biochemical defects in mitochondrial respiration and oxidative phosphorylation caused by the 3243-nd point mutation in the mitochondrial DNA do not affect the sperm motility.

**Support** none

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**W-025 Male infertility**

Prevalence of diabetes mellitus in male partners of couples attending a tertiary fertility center

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**Background** WHO reports that prevalence of diabetes has been on the rise worldwide and especially in countries like India, there is a phenomenal increase in the number of people diagnosed with Diabetes each year. In 2000 the number was 31.7 million which is expected to multiply by 2010 to 79.4 million. Diabetes has been implicated as a contributory factor associated with male infertility in recent times, with many studies showing a positive correlation between diabetes and infertility. Male reproductive function is affected by diabetes at various levels as proven by many animal models. So far studies to estimate the prevalence of this disease among male infertile patients are very few and according to our knowledge prevalence studies from Indian population which has significant amount of diabetic population, are nil.

**Aim** The aim of the study was to estimate the prevalence of diabetes in infertile male population attending our fertility center and to study the correlation between the fasting blood sugar levels and semen parameters like semen volume, sperm concentration, motility and morphology as analyzed by WHO standards.

**Materials and Methods** Retrospective analysis of the semen analysis reports of infertile male patients who attended the clinic between October 2007 to October 2009 were analyzed. The patients included in the study were within the age group of 28–47 years. A total of 923 patients were included.

**Results** Out of 923 patients who had undergone semen analysis for evaluation of infertility, 54 patients had elevated fasting blood glucose levels. The prevalence of diabetes in the population studied was 5.8%. Among them, the prevalence was more in the age group of 30–34 yrs (n = 17) who constituted 31.5% of the total diabetic population, followed by the age group of 35–39 years (n = 15) who formed 28.7% of the diabetic population. The mean fasting blood sugar level among the 30–34 years age group was 169.67 and in 35–39 years group it was 165.86. Semen volume varied considerably with increased blood glucose levels showing an inverse relationship where as the sperm concentration, sperm motility and sperm morphology did not correlate with altered blood sugar levels.

**Conclusion** Fasting blood sugar levels are inversely proportional to semen volume. Elevated blood sugar levels per se do not appear to have significant correlation with sperm concentration, motility or morphology.

**W-026 Male infertility**

Suboptimal semen parameters: Is ICSI the only option?

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**Introduction** The introduction of intracytoplasmic sperm injection (ICSI) has revolutionised the treatment of severe male factor infertility. It has demonstrated that neither the number of sperm or motility rates have any influence on the fertilization rate following ICSI. However, the use of ICSI in cases where it is not indicated is time-consuming and costly. Furthermore, ICSI is a technique which is still not fully safety evaluated. The rates of ICSI use have dramatically increased in recent years without a mirrored increase in male factor infertility. This raises concerns and from this perspective we aimed to compare outcomes of conventional IVF with ICSI in a subfertile male population where both techniques could be used.

**Methods** A retrospective data analysis of a total of 268 IVF and ICSI cycles was performed. We identified females with a normal FSH (< 9.4 IU/L) and age less than 40 years old. On the basis of total sperm count and motility (jaculate) we identified the following groups: Group I Count ≤ 20 ml/m; motility < 50%, Group II Count ≤ 20 ml/m, motility > 50%, Group III Count > 20 ml/m; motility < 50% motile. The lower cut-off for sperm count was 5 million/ml and for motility was 10%. The following parameters were analyzed: type of laboratory procedure (IVF, ICSI), fertilization rates, positive hCG rates and clinical pregnancy rates per oocyte recovery.

**Results** In IVF Group I, (n = 25), vs ICSI Group I, (n = 82), outcomes of treatment were better in the ICSI group, fertilization (43% vs 58%; p = 0.0001), positive hCG (28% vs 53%; p = 0.03) and CPR (20% vs 46%; p = 0.02). In Group II (IVF, n = 39) and (ICSI n = 32), the fertilization, positive hCG and CPR results were 57% vs 59%, 51% vs 34%, and 36% vs 28%, respectively. A total of 61 patients were identified in IVF Group III vs 29 patients in ICSI Group III. In IVF Group III, the fertilization rate was 52%, positive hCG 41% and the CPR was 32%, compared to a fertilization rate of 52%, a positive hCG of 38% and a CPR of 31% in ICSI Group III.

**Conclusion** Our data shows that when both parameters (count and motility) are suboptimal (≤ 20 million or < 50%), ICSI should be the preferred choice. When at least one parameter is within normal limits there is no benefit of offering ICSI over IVF. A re-evaluation of indications for ICSI should be considered!

**W-027 Male infertility**

HSL knockout (KO) male mice are sterile and have over-expressed scavenger receptors class B (SR-BI), activated cell signaling pathways and disrupt lipid raft microdomains in testis

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In testis, SR-BI mediates selective uptake of cholesterol esters from LDL, which are hydrolyzed to unesterified cholesterol by HSL. This enzyme is critical since HSL KO male mice are sterile and cells accumulate cholesterol in the cytoplasm. SR-BI is anchored to the lipid rafts plasma membrane, that are microdomains enriched with cholesterol and sphingolipids involved in cholesterol traffic and major platforms for initiation, propagation and maintenance of signal transduction events.

**Objectives** To determine effects of the lack of HSL in testis mice on scavenger receptor class B expression, lipid rafts and cell signaling pathways.

**Methods** WT and HSL KO mice testis frozen or embedded in paraffin to study protein expression and immunolocalization. Seminal fluid obtained from epididymis for spermatozoa counts and motility. Mice testis lipid rafts isolated by sodium carbonate extraction and sucrose gradient fractionation.

**Results** HSL KO mice testis presented altered spermatogenesis associated with decreased sperm counts, sperm motility and in-
W-028 Male infertility
Is there an indication for testicular sperm extraction in men with severe oligoasthenoteratozoospermia before intracytoplasmic sperm injection?

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Introduction
Performing intracytoplasmic sperm injection (ICSI) in patients with severe oligoasthenoteratozoospermia (OAT) syndrome can be challenging, because a selection of normal spermatozoa is impaired by the extremely low amount of sperm from a native semen sample. To avoid absence of injectable sperm on the day of ovum pick up stand-by-testicular-sperm-extraction (TESE) has been used in daily routine, demanding a considerable amount of time, manpower, financial burden and emotional implications for the patients. We conducted this study to evaluate clinical data of severe OAT patients to predict if a testicular biopsy is necessary or if native semen samples at the day of ovum pick up will be sufficient for ICSI. This may help to omit stand-by-TESE.

Materials and Methods
From 2004 to 2008 we retrospectively analyzed clinical data of 91 stand-by-TESE patients including therapy, semen parameters, hormone values (FSH 15.3 ± 9.1 U/l), testis size, age of both partners (men 35 ± 6, women 32 ± 5 years) and embryo quality. Correlation and prediction of 2 semen analysis before the day of ovum pick up and semen parameters on the day of ICSI were tested as well as the significance of sperm concentration and motility for fertilisation and pregnancy rates.

Results
Sperm concentration and motility of the examinations before ovum pick up correlated with the semen analysis on the day of ICSI (p = 0.013, p < 0.0001). Sperm concentration on the day of ICSI correlated with the number of fertilised oocytes (p = 0.058). Ejaculates with a sperm concentration < 0.1 mill/ml (group 1, n = 49) showed a reduced percentage of forward motile sperm (p = 0.0001) in comparison to semen samples with a sperm concentration between 0.1 and 1 mill/ml (group 2, n = 32) with 13 % versus 25 %, respectively. Clinical pregnancy rate in group 1 (5/44) was significantly lower (p = 0.038) than in group 2 (9/23). Only 5 testicular sperm extractions on the day of ICSI were necessary.

Conclusion
After the evaluation of the second semen sample the therapy can be determined. If motile sperm are present in the native ejaculate no (stand-by-) TESE is necessary. A Sperm concentration < 0.1 mill/ml is correlated with lower clinical pregnancy rates. The result of this study helps to save time and financial resources and improve counseling of patients about their realistic chances of fertilisation before starting treatment.

W-029 Male infertility
Clinical efficacy of Intracytoplasmic morphologically selected sperm injection (IMSI) in infertile patients with severe male factor or poor prognosis

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Objective
Intracytoplasmic morphologically selected sperm injection (IMSI) is a high magnification light microscopy imaging method used increasingly in the field of in vitro fertilization (IVF) to select sperm for intracytoplasmic sperm injection (ICSI). The conventional magnification displays only simple sperm morphology and not detailed functional characteristics such as fine morphology and nuclear abnormality of sperm. To overcome this drawback, Bartoo et al. introduced a modified IMSI procedure. A sophisticated advancement of ICSI is IMSI. The only difference from ICSI is that it carefully selects the best quality sperm by motile sperm organellar morphology examination (MSOME) than in the conventional ICSI method. The aim of the present study was to determine whether subtle sperm morphological characteristics affect the outcome of intracytoplasmic sperm injection (ICSI).

Design
Comparative analysis of pregnancy rates resulted from the transfer of ICSI technique versus IMSI technique based on morphological selection of spermatozoa with normal nuclei.

Materials and Methods
This study had been performed on 97 cycles, 66 cases (68.0%) of conventional ICSI cases and 31 cases (32.0%) of IMSI cases, from our laboratory in May–October, 2009. Couples with severe oligoasthenoteratozoospermia or poor prognosis (≥ 3 years of primary infertility and ≥ 2 times of IVF failures) were enrolled for this study, and they were then randomized for microinsemination treatment.

Results
Average age of female patients was 35.43 ± 3.88 years old and number of attempted IVF cycle and aspirated oocytes per cycles were 2.30 ± 1.32 cycles and 11.31 ± 6.80, respectively. In total of 97 cases, 14 of 66 IMSI cases (21.2%) resulted in pregnancy while 13 of 31 ICSI cases (41.9%) also resulted in pregnancy (p < 0.05).

Conclusion
Based on the above results, we suggested that the application of IMSI in infertility patients with severe male factor resulted higher clinical pregnancy rate than conventional ICSI. Also, despite their initial poor reproductive prognosis, patients with two or more previous failed attempts benefited the most from IMSI.

Support
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senting 12 proteins were identified by mass spectrometry. Ficolin-3, and α antichymotrypsin (AAT) showed significant increase (q; 2.0 and 1.6-fold, respectively, p < 0.001, false discovery rate q of 0.002-0.2) compare to control while vitamin D binding protein (VBP) showed a 2.0-fold decrease in KS. A comparison between the individual spot volumes obtained with the Dige method for VBP and a corresponding 2D SDS-PAGE western blot showed a significant correlation between methods (r = 0.63; p = 0.05). Multiple discriminate analysis showed a significant direct association between isoforms of VBP and AACT with serum testosterone. In addition, significant inverse associations were observed between VBP, ficolin-3, AACT and serum total inhibin. No plasma proteins unique to KS were identified. Independent validation of the changes in spot volumes of these proteins in plasma by specific ELISAs was not observed due to the presence of multiple isoforms of each of the proteins. Subsequent 2D Western analysis has confirmed isoform heterogeneity and provides an explanation for the differences observed between quantitative methods.

Conclusion Plasma protein differences observed between KS and controls are attributed in part to the altered endothelial milieu (as seen with the wide range in serum testosterone levels found in these patients), and to the altered testicular function in KS. The inverse association between total inhibin and these three proteins suggests a link between the regulation of their plasma levels and gonadal function.

**W-031 Male infertility**

**Influence of low and high population density on mouse sertoli cells and spermatozoa**

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**Aims**

Human typically face in the modern, developed world with stressors that originate largely from social and interpersonal interactions. Epidemiological data suggest that stress is linked to the development of metabolic disease and infertility. The importance of sertoli cells in spermatogenesis has been demonstrated also quantity and quality of spermatozoa are two important factors for fertility. Whereas dysfunction of sertoli cells and spermatozoa are often associated with changes in reproductive system function, in the present study the effects of low and high population density on number of sertoli cells, shape, and number of spermatozoa were investigated.

**Methods**

30 male mice were divided into 3 groups. Control group (5 mice/cage), group 1 (10 mice/cage) and group 2 (15 mice/cage) and kept for 2 months. The mice were anesthetized with an injection of ketamine and xylazine. Spermatozoa were harvested from the caudae epididymis. The samples of testes were removed and processed for light microscopic studies and the numbers of sertoli cells were investigated. The caput epididymis was prepared for Transmission Electron Microscopic (TEM) studies and the shape of spermatozoa in it was investigated. The data has been compared using statistical methods (SPSS, ANOVA, Kruskal Wallis test and p < 0.05).

**Results**

The results showed that the numbers of sertoli cells in group 2 have significantly decreased as compared with group 1 (p < 0.004), but the differences in other groups were not significant. Moreover the numbers of collected spermatozoa in group 2 as compared with group 1 were shown significant decrease (p < 0.004); whereas the differences in other groups were not significant. In TEM studies the spermatozoa in group 2 have residual bodies as compared with control group and group 1.

**Conclusions**

These results suggest that crowding stress can affect on number of sertoli cells, number and shape of spermatozoa in male mice.

**W-032 Male infertility**

**Intracytoplasmic morphologically selected sperm injection benefits on severe male factor profiled according to the new 2010 WHO reference values**

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**Introduction**

The development of the morphologically selected sperm injection (IMSI) demonstrated that a profound morphological investigation of the spermatozoon enables outcome improvement. The aim of this study was to compare IMSI efficacy on male factor infertility cases based on WHO guideline from 1999 and WHO guideline from 2010 reference values.

**Methods**

Enrolled patients were split into 2 groups: IMSI-Group (n = 250), which underwent IMSI; and Control-Group (n = 250), which underwent intracytoplasmic sperm injection (ICSI). Fertilization, percentage of high-quality embryos, pregnancy, implantation and miscarriage rates were compared among the groups. In a further analysis, two different groups of patients were analyzed separately:

1. Oligoastenozoospermic patients according to the WHO reference values from 1999 and
2. Oligoastenozoospermic patients according to the suggested reference values in 2009

Regression analysis was performed to assess the influence of IMSI on treatment outcomes.

**Results**

No significant differences were observed between the IMSI-group and ICSI-group for any evaluated variable, except for fertilization rate, which was significantly higher in IMSI group (68.0% vs 73.0%; p = 0.013). When patients were considered oligoastenozoospermic according to the WHO guideline from 1999, a positive influence of the IMSI on the fertilization rate was also observed (OR: 1.3; CI: 0.7–2.1; p = 0.043). No impact of IMSI on the other variables was observed. Regarding oligoastenozoospermic patients according to the suggested reference values from 2009, a close relationship between the IMSI and fertilization rate (OR: 4.3, CI: 2.2–6.4; p = 0.004) was noted. Moreover, considering these new reference values, the use of morphologically selected sperm was deterministic to the likelihood of implantation (OR: 2.6, CI: 1.2–5.7; p = 0.013) and pregnancy (OR: 1.67, CI: 0.8–3.0; p = 0.045) occurrence.

**Conclusion**

Regardless of the infertility cause, IMSI procedure significantly improves the fertilization rate. Regarding male infertility factor, we observed that the more severe the male factor, higher the improvements on IMSI outcomes, especially when diagnosis was based on the recently suggested reference values. With the introduction of new cut-off points, oligoastenozoospermic men, who may be responsible for the subfertility of the couple, could be more accurately identified, treated with IMSI, resulting in improved treatment outcomes.

**Funding**

none

**W-033 Menopause**

**Assessment of consumption of suitable diet in prevention from complications of menopause in women living in Borugerd City, Iran**

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This study is a descriptive correlitive one which will be done to determine consumption of appropriate diet in prevention from complications of menopause in three hundred eligible menopausal women in Borugerd City. Questions of this research will be proposed on the basis of its goals.

The tool of collecting data is interview form that researcher will do by face-to-face interview with subjects. Sampling method will be cluster. The descriptive and deductive statistic and computer software SPSS will be used to analyze data. Considering that this research is being performed now; the results of the research will be sent consequently after finishing the research if the article is accepted.
W-034 Menopause
Menopausal status in breast cancer patients determines long-term sexual desire

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Introduction Chemotherapy and/or ovarian suppression by endocrine therapy in young breast cancer patients is frequently associated with abrupt menopause with negative effects on sexual functioning. Very little is known about the long-term prevalence of hypoactive sexual desire disorder (HSDD) in these patients. The Sexual Interest and Desire Inventory-Female (SIDI-F) was developed as a clinician-administered assessment tool to quantify severity of HSDD. Menopausal symptoms and disorders of sexual desire in breast cancer patients have been explained by the disruption of hormone production in granulosa cells of the growing follicle-luteal cycles with lower circulating levels of estrogen and/or androgens. The grade of depletion of the primordial follicle pool can be quantitatively characterized by the measurement of anti-Müllerian-Hormone (AMH) in serum.

Methods Controlled, cross-sectional study: 34 patients (mean age 42 years) had breast cancer primarily treated with surgery and chemotherapy and eventual endocrine therapy: No adjuvant endocrine therapy (n = 18) or adjuvant endocrine therapy (n = 16), 13 patients (mean age 40) had breast surgery due to benign breast disease. We measured estradiol, testosterone and AMH in serum.

Results Mean SIDI-F scores were similar in the breast cancer group and in the benign breast disease group. Mean AMH levels were significantly lower in the breast cancer group (0.1 ng/ml) as compared to the benign breast disease group (0.77 ng/ml). Subgroup analysis revealed that differences of the mean SIDI-F scores according to the actual endocrine were not statistically significant. In contrast, in breast cancer patients with the induction of menopause by chemotherapy or endocrine therapy the SIDI-F scores were significantly lower (30.7) compared to breast cancer patients with normal or oligomenorrhea (40.4). Mean estradiol and testosterone levels were not statistically different in these two groups.

Conclusion Chemotherapy in breast cancer patients does not significantly impair long-term sexual desire as compared to patients with benign breast disease. Adjuvant endocrine therapy with gonadotropin releasing hormone agonists in breast cancer patients has a negative impact on sexual desire. Patients with menopause, permanently induced by chemotherapy or actually induced by gonadotropin releasing hormone agonists, show significantly reduced sexual desire as compared to menstruating patients with past chemotherapy.

W-035 Menopause
Age at natural menopause and menopausal symptoms among Saudi Arabian women in Al-Khobar

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Menopause is physiological event, experienced as a long process of climacteric change (transition phase that is immediately prior to and after menopause, when clinical, biological, and endocrinological symptoms of menstrual cessation commence), occurring universally in all women who reach midlife. The timing of menopause as well as women’s experience of menopausal symptoms varies between populations and within populations. Age of menopause and climacteric symptoms have been extensively studied in the developed world as well as in developing countries; but there have been few systematic studies of menopause in Arab countries. The current study was conducted in 2003 among Saudi Arabian women in the city of Al-Khobar in the East of Saudi Arabia. Biocultural and lifespan perspective were used to assess age at natural menopause and the prevalence of menopausal symptoms. The objectives of the study are:

1. To assess the mean and median age at natural menopause
2. To detect factors that might contribute significantly to a more rapid decline to menopause
3. To determine factors that significantly affect the prevalence of menopausal symptoms that Saudi Women’s experience; and
4. To assess the relationship between women’s perceptions and attitudes toward the menopausal event. This study also examines the differences in women’s attitudes toward menopause, depending in whether these women were experiencing, or had already experienced natural menopause.

In this cross-sectional study, a face-to-face questionnaire was administered to 200 Bedouin and Hadar Saudi Arabian women. The mean age of the sample ranged from 40 to 55 years. The finding of this study indicated that while age at natural menopause varied within and among the menopausal factors (anthropometric measures, socio-demographic status, reproductive history, and life style); the only factors that were significantly associated with age at natural menopause were women’s weight, marital status, and employment status. The finding also indicated that the respondent’s marital status and number of children were significantly associated with the prevalence of menopausal symptoms. Women’s attitudes toward menopause varied based on their menstrual status and ethnicity. And there was a significant association between the respondents total menopausal symptoms reported and their attitudes toward the menopausal event and the women’s educational level.

W-036 Menopause
The risk factors of menopausal metabolic syndrome in perimenopausal women

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Aim To study the risk factors of menopausal metabolic syndrome in perimenopausal women.

Methods Population study of randomly chosen 512 women at the age of 45–55 years and longitudinal clinical study of 370 women (191 natural menopausal and 179 surgical menopausal) during 5 years after menopause.

Results The BMI ≥ 25 kg/m² was registered in 60.2%, menopausal metabolic syndrome was registered in 11.3% of women at the age of 45–55 years. Increased risk of obesity was observed among participants with uterine leiomyoma (odds ratio [OR] = 1.8; 95% confidence interval (CI): 1.2, 2.7; p < 0.05) and menopausal metabolic syndrome more often was registered among participants with uterine leiomyoma (OR = 1.5; 95% CI: 0.8, 2.6; p > 0.05). Assume associations of uterine leiomyoma with atherogenic risk factors. Menopausal metabolic syndrome was positively correlated with heredity on obesity, hypertension or diabetes mellitus (r = 0.2; p < 0.05), impaired glucose tolerance (r = 0.3; p < 0.05), BMI (r = 0.3; p < 0.05), increased BMI after menopause (r = 0.1; p < 0.05), hypertension (r = 0.1; p < 0.05), thyroid diseases diagnosed at ages more then 45 years (r = 0.1; p < 0.05).

Conclusions Our data show that, the risk of development of menopausal metabolic syndrome in perimenopausal women raises at a combination of uterine leiomyoma, surgical menopause, thyroid diseases diagnosed at ages more than 45 years, heredity on obesity, hypertension or diabetes mellitus, glycemia > 5.6 mmol/l, BMI ≥ 25 kg/m² and progressing increase of body weight after menopause.

W-037 Menopause
Premature ovarian failure

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Objectives The objectives of this study are to investigate different epidemiology characteristic, and mechanisms of premature ovarian failure.

Results The incidence of premature ovarian failure is around 1–3%. This pathology occurs in young women who often wish to
Human follicular fluid as a cache for possible biomarkers

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Human fertility is a growing issue of the mankind and in vitro fertilization (IVF) is still not perfected technique and bears some severe complications, such as ovarian hyperstimulation syndrome (OHSS). The use of different -omics technologies could reveal some hidden secrets of the reproductive process and help to improve current IVF techniques by the means of avoiding possibly life-threatening conditions and increasing birth per embryo transfer rate. Human follicular fluid (HFF) provides a microenvironment of the oocyte and contains many molecules that may influence oocyte quality.

Firstly, we focused on comparison of protein profiles of HFF and blood plasma and the results of this study have shown the importance of innate immunity and angiogenesis co-regulation in the preovulatory follicle of women undergoing hormonal stimulation for IVF. To extend these results we monitored cytokine/chemokine profiles of HFF and blood serum. Finally, the analysis was performed to search for a diagnostic/prognostic biomarker(s) of OHSS in the HFF.

The body fluids for protein profiling were depleted of the 12 most abundant proteins of human blood plasma using IgY-12 immuno-affinity system (Beckman Coulter). For the protein profiling we have used 2 complementary techniques: 2D-electrophoresis and 2D liquid chromatography. Some of the differentially expressed proteins were validated using independent techniques and non-depleted samples. For the tailored quantification of cytokines and chemokines was used the Lumiplex XMAP multiplexing technology together with Milliplex cytokine/chemokine assay kits (Millipore).

The depletion of the 12 most abundant proteins successfully allowed to access middle- or low- abundant proteins of HFF and plasma. The proteomic data demonstrated the importance of innate immune system, angiogenesis and blood coagulation in preovulatory follicles. The co-regulation observed in these pathways was further highlighted at the level of cytokine regulation. Several individual proteins or cytokines indicated quantitative changes related to OHSS but only their combination might be promising for further validation.

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W-041 Ovarian deficiency
Copy number variants in premature ovarian failure and ovarian dysgenesis

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Premature ovarian failure (POF) is a heterogeneous group of disorders with amenorrhea and high serum gonadotropins in women with less than 40 years. Ovarian dysgenesis (OD) which is characterized by the loss of follicles before puberty describes the most severe POF outcome. Although a multitude of different factors including non-genetic as well as genetic are known to play a role in the development of POF and OD, the underlying etiology remains unsolved in the majority of cases. In the last years array-CGH was found out to be a very useful tool in the identification of candidate genes in different conditions. Therefore, we performed array-CGH analysis by using high-resolution Agilent oligonucleotide arrays in a total of 74 POF and OD patients and identified 44 private losses and gains potentially causal for POF.

W-042 Ovarian stimulation
Prevalence of low responders in the pilot batch of IVF stimulated cycles at the IVF Center, National Hospital, Abuja, Nigeria

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Context It has been observed that the majority of clients in Nigeria seek assisted conception in their late thirties, forties and even fifties in terms of age, raising questions about their ovarian responsiveness. Identifying low responders is of critical clinical importance, as these patients require specialized management to optimize the number and quality of oocytes that may be available for assisted reproductive technology procedures.

Methods It was a retrospective study. The pilot batch of clients for the new IVF Center were recruited and treatment cycles occurred between July and August 2006. Controlled ovarian hyperstimulation was performed with varying doses of human menopausal gonadotrophin depending on the client’s age. Low responders were identified using the following criteria: a peak estradiol level of less than 500 mIU/L or the production of less than four mature oocytes at the time of human chorionic gonadotrophin administration.

Results There were a total number of 17 clients. One client did not proceed to controlled ovarian stimulation due to medical complication of diabetic ketoacidosis, leaving 16 to be analyzed. Only one case of poor ovarian response was identified in the group of clients. This was a 34 year old woman with estradiol levels at 47.89 mIU/L on day 8 of stimulation with HMG, 159.3 on day of HCG administration, and 60.79 on oocyte retrieval day. This subject also had less than four mature oocytes on the day of human chorionic gonadotrophin administration.

Conclusion The prevalence of low responders in this pilot batch of cycles is low at 6.25%. However, the limitation of this statement may be due to the small sample size. Controlled ovarian hyperstimulation was performed with varying doses of human menopausal gonadotrophin depending on the client’s age. Large-scale trials are needed to assess the magnitude of this phenomenon in our society, in order to proffer solutions where appropriate.

W-043 Ovarian stimulation
Unexended decreases in serum estradiol prior to the administration of human chorionic gonadotrophin do not influence the outcome of in vitro fertilization

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Objective The purpose of this study was to evaluate the effects of an unexpected drop in serum estradiol prior to human chorionic gonadotrophin (hCG) administration on the outcome of in vitro fertilization (IVF).

Design Retrospective cohort study.

Setting A university hospital.

Patients The study population included patients who underwent an unexpected drop in estradiol without coating in IVF cycles using a long agonist protocol and an antagonist protocol (n = 138), with an equal number of controls (n = 138).

Interventions Patients were stratified by the degree of decreased estradiol level and compared with controls. In addition, patients were divided into two groups (i.e., those with decreased estradiol, with or without gonadotropin dose reduction) and compared with controls.

Main Outcome Measure Metaphase II (MII) oocyte rate, fertilization rate, clinical pregnancy rate, and live birth rate were compared.

Results The number of metaphase II oocytes (5.4 ± 2.4 vs 5.5 ± 4.6) and the number of fertilized oocytes (7.3 ± 5.0 vs 7.3 ± 5.1) were similar in patients with decreased estradiol and in controls, respectively. There were no differences in clinical pregnancy rates (32.6% vs 40.6%) or live birth rates (29.7% vs 39.1%) between the study and control groups. The magnitude of estradiol reduction did not affect IVF outcomes. When the study groups were divided into patients with decreased estradiol for whom the gonadotropin dose was reduced or maintained, there were no differences in clinical pregnancy rate or live birth rate between the control and decreased estradiol groups.

Conclusion Unpredictable drops in serum estradiol prior to hCG administration do not affect the success of IVF cycles using long agonist and antagonist protocols.

W-044 Ovarian stimulation
Comparison of endometrial development following short course Anastrozole and Clomiphen citrate for ovulation induction in unexplained infertility

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Objective To compare the effect of anastrozole with clomiphen citrate on endometrial growth for ovulation induction in women with unexplained infertility.

Methods A total of 41 unexplained infertile women who failed to get pregnant following clomiphen citrate treated cycles were studied in 2 groups. The patients were selected among patients referred to university hospital. Anastrozole dose of 1 mg/day (n = 19) was given from days 3–5 of menstrual cycles followed by hMG75 u/day from days 6–8. Clomiphen citrate a dose of 100 mg/day (n = 22) from days 3–7 followed by human menopausal gonadotrophin (hMG)/75 u/ml administered every day starting on day 6. When the leading follicle was more than 18 mm in diameter a single dose of urinary hCG was administered to stimulate ovulation. Endometrial thickness was measured by transvaginal sonography before stimulation and before hCG injection.

Results There were no differences in demographic characteristics between groups. Endometrial thickness at the time of hCG administration was not significantly higher in Anastrozole group (4.82 ± 1.98 mm vs 3.7 ± 2.34; p-value = 0.115) than clomiphen group.

Conclusion Short course (Day 3) anastrozole and (Day 5) clomiphen citrate in association with hMG have equally the same effects on endometrial thickness in inducing ovulation in patients with unexplained infertility.

W-045 Ovarian stimulation
A minimal stimulation protocol with simplified monitoring for superovulation and intrauterine insemination

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Objective To evaluate the efficacy and cost of a simplified superovulation regimen in infertile patients undergoing intrauterine insemination (IUI).
Design Analysis of 170 minimal stimulation and 130 conventional HMG cycles.

Setting Infertility Clinic, Shathy Hospital for Women, Alexandria.

Patients 180 women with either unexplained infertility or ovulatory dysfunction who ovulated with clomiphene citrate but failed to conceive.

Interventions 90 patients underwent IUI using a simplified superovulation regimen that included clomiphene citrate 100 mg on cycle days 3 through 7 and HMG 75IU on cycle days 5-7. A control group (n = 90) received the standard HMG protocol. In both groups many patients received more than one treatment cycle.

Main Outcome Measures Clinical pregnancy rate, medication and monitoring costs.

Results There was no difference in the couples of the 2 stimulation protocols regarding age, duration and type of infertility, as well as, cause of infertility. The monitoring costs and total ampoules of HMG required differed significantly (4 ampoules for minimal stimulation versus 14 + 3.2 in the conventional HMG). The clinical pregnancy rates per cycle were comparable (7.78% vs 11.11% for HMG). No patients were admitted for ovarian hyperstimulation.

Conclusion Minimal stimulation for IUI is less expensive than conventional HMG stimulation and minimizes monitoring and patient discomfort. The comparable pregnancy rates and decreased medication costs of minimal stimulation justifies further evaluation of its role in the treatment of infertility.

W-046 Ovarian stimulation Recombinant HCG microdose supplementation effects in ovarian stimulation protocol: A prospective randomized trial

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Introduction The objective in this study was to compare the outcomes of controlled ovarian stimulation (COS) in patients < 35 years old and ≥ 35 years old undergoing intracytoplasmic sperm injection (ICSI) cycles with exclusively recombinant FSH (r-FSH) or in association with recombinant hCG (r-hCG) microdose.

Materials and Methods We evaluated the outcomes of COS in 320 patients < 35 y-old and 68 patients ≥ 35 y-old of age. In both groups, patients were divided into a control group (n = 160 < 35 y-old and n = 34 ≥ 35 y-old) and a Microdose group (n = 160 < 35 y-old and n = 34 ≥ 35 y-old). Down regulation was achieved trough leuprolide acetate. In control-groups follicular growth was stimulated with a FSH (225 IU daily). In Microdose-groups, when at least one follicle ≥ 14 mm was observed, the r-FSH dose was reduced to 75 IU. In this case, r-hCG microdose was administered subcutaneously (7.7 µg) until the day of ovulation triggering. The number of metaphase II (MII) oocytes, percentage of high quality embryos, fertilization, pregnancy, implantation and miscarriage rates were evaluated.

Results In patients < 35 y-old, a higher number of MII oocytes was observed in the Microdose-group (10.2 ± 7.19 vs 8.31 ± 7.01; p = 0.019); however no differences were observed regarding the other evaluated variables. In patients ≥ 35 y-old the Microdose group showed a higher fertilization rate (75.9% vs 60.7%; p = 0.036), and a higher percentage of high-quality embryos (66.5% vs 51.1%; p = 0.018). Moreover, a trend for a higher implantation rate in Microdose-group was noted (12.3% vs 6.4%; p = 0.085), however this most likely did not reach statistical significance because of insufficient number of cycles evaluated in this trial.

Conclusions The increased number of MII oocytes, observed in group of patients < 35 y-old treated with the r-hCG microdose, suggests that the association of r-hCG and r-FSH may prevent the decrease on estradiol concentration after GnRH antagonist administration, which in turn, may significantly increase the number of mature retrieved oocytes. For patients ≥ 35y-old the use of hCG microdose was able to improve both fertilization and embryo quality, suggesting an important role of LH during the latter phase of oocyte development. In conclusion, our data confirm that the recombinant hCG microdose is an efficient source of LH activity for both younger and older patients, and this strategy can be used to reduce the FSH amounts required in COS protocols.

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W-047 Oocyte Ageing induced changes in some epigenetic markers and related proteins in mouse oocytes

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In the mammalian female ageing is an important factor related to the decrease of fertility. Various alterations have been described in the aged ovulated oocyte. Nevertheless, alterations occurred during the germinal vesicle stage (GV) are scarce. We have used young (3 months old) and aged (12–18 months old) mouse females to analyze some changes that take place in the nucleus of GV. Aged GV showed numerous changes in their chromatin structure, and the 25% of oocytes presented abnormal chromatin forms. Aged GV also show changes in the Histone H3 methylation, in Histone H4 methylation and in Histone H4 acetylation. Furthermore, it was observed that some proteins related to these methylation and acetylation phenomenae were modified in aged GV. This is the case of protein SIRT2 (related to H4 acetylation) and protein HP1B (related to Histone H3 methylation). All these modifications are obviously inherited by the ovulated oocyte and could be a negative factor for the achievement of fertility.

W-048 Oocyte Molecular profiles of ovarian folliculogenesis

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Recently, assisted reproductive medicine has developed noticeably and provided treatments for lots of infertile couples. However, more advanced technologies are required for more severe infertility such as premature ovarian failure and ovarian failure due to adjuvant therapies for cancer. Ovarian tissue cryopreservation followed by in-vitro growth of isolated follicles is a feasible method for those patients. Ovarian folliculogenesis contains the serial growing stages from primordial follicles to graafian follicles in mature ovaries. The middle and late stages are regulated by gonadotropins secreted from the pituitary. Close communication among follicle cells including oocytes, granulosa and theca cells is coordinated for follicle growth. On the other hand, primordial follicles start to grow independent of the presence of gonadotropins. Crucial factors must regulate this growth phase as well as the gonadotropin dependent stages. To develop a culture system for early growing follicles we conducted a research project to identify factor(s) necessary for the early stages of folliculogenesis. For this purpose we adopted DNA microarray analysis using mouse ovaries at ages of 7, 10, 13, 16 and 19 days, because juvenile mouse ovarian follicles grow concomitantly and develop according to chronology. As a result, we found GDP-9 and BMP-15 showed strong intensity in 7-day-old mice and gradually decreased until 19 days old. SCF, AMH and PDGF showed relatively high expressions, while IGF-1 and FGF-8 showed low intensity. Addition of high intensity factor(s) to the culture medium may be effective for in-vitro growth of early-stage follicles.
**W-049 Oocyte**

**Human metaphase oocytes from antral and pre-ovulatory follicles exhibit comparable developmental competence**

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**Introduction** In vitro maturation (IVM) cycles primed with HCG, in addition to immature oocytes, is possible to retrieve mature oocytes from antral follicles of 11–13 mm. These mature oocytes give rise to embryos which appear to implant with a relatively high rate, challenging the notion that a more prolonged follicle growth is essential for oocyte developmental competence. In this pilot study, we compared the outcome of mature oocytes derived either from antral follicles of HCG-primed IVM cycles or preovulatory follicles of standard controlled hyperstimulation (COH) cycles.

**Materials and Methods** IVM was performed in cycles involving HCG administration when the leading follicle was 11–13 mm. COH was obtained by a long protocol including agonist suppression, rFSH stimulation, and HCG administration when the leading follicle was >18 mm. In all treatments, patient age was <39 years. As prescribed by law, maximum 3 oocytes classified as mature at the time of retrieval were selected for each treatment cycle. Fertilization was achieved by ICSI. All embryos were transferred without any selection, in compliance with law. A Fisher's exact test adjusted for age was adopted to compare statistically percentages of fertilization, embryo quality, and implantation between the 2 groups.

**Results** In the IVM and COH groups, respectively, 256 and 2515 oocytes were microinjected. The percentage of normal fertilization was 81.6% (209/256) in the IVM group and 77.6% (1951/2515) in the COH group (p = 0.103). 204 embryos (204/209, 97.6%) were obtained and transferred in IVM cycles, with a frequency of cleavage comparable (p = 0.145) to the COH group (1871/1951, 95.9%). The proportion of high quality embryos, i.e., those with less than 20% fragmentation, was not statistically different (89.5% and 85.2%, respectively) in the IVM and COS treatments (p = 0.703). Implantation rates per inseminated oocyte were 11.7% in the IVM group and 6.7% in the COH group, while implantation rates per embryo transferred were 14.7% and 9.0%, respectively (p > 0.05).

**Conclusions** The present data indicate that mature oocytes retrieved in IVM cycles from antral follicles after HCG priming can fertilize, develop, and implant with rates comparable to those of oocytes from preovulatory follicles. This might suggest that the latest stages of follicular development are not critical for the acquisition of oocyte developmental ability. Further studies are warranted to test this hypothesis.

**W-050 Oocyte**

**Influence of social stress on DNA fragmentation rate in mouse oocyte**

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**Aims** Increase of social stress following progress in technology can be an important factor that can cause emotional, physical disorders and infertility. Since women are more likely to experience a clinically significant depression than men and depression in women is often associated with changes in reproductive system function, in the present study the effects of social stress on mouse oocyte DNA fragmentation rate were investigated.

**Methods** 70 female mice were divided into 3 groups. Control group (5 mice/cage in 4 cages), group 1 (10 mice/cage in 2 cages) and group 2 (15 mice/cage in 2 cages) and kept for 1 month. Female mice in 3 groups were superovulated with PMSG and hCG and anesthetized by cervical dislocation 15 h after hCG injection. In 3 groups the oocytes were collected from mouse oviducts by flushing the Ampula. After fixation with 4% paraformaldehyde, the grades I and IV of oocytes were selected and finally they were stained with TUNEL method for detection of DNA fragmentation rate. The data has been compared using statistical methods (SPSS, ANOVA, Kruskal Wallis test and p < 0.05).

**Results** The results showed that the DNA fragmentation rate of grade I and IV oocytes in group 1 and group 2 were significantly increased as compared with control group (p < 0.013, p < 0.011); but the differences between groups 2 and 1 were not significant. The DNA fragmentation rate of grade I oocytes in group 1 were significantly increased as compared with control group (p < 0.008); whereas in group 2 as compared with group 1 were showed significant increased (p < 0.001), and the differences between group 2 and control group were not significant. The DNA fragmentation rate of grade IV in group 1 and group 2 as compared with control group and between groups 2 and 1 were significantly increased (p < 0.004, p < 0.001, p < 0.005).

**Conclusions** These results suggest that crowding stress can change the apoptotic rate of grade I and IV oocytes in female mice.

**W-051 Oocyte**

**Novel approaches to study the mitochondrial glutathione redox potential (EmGSH) non-invasively in mammalian oocytes: Grx1-roGFP2 as biosensor**

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**Introduction** Over 90% of the cellular reactive oxygen species (ROS) in mammalian cells are produced by the mitochondria. Mitochondria are abundant in oocytes and it has been suggested that ageing, suboptimal culture and dysfunctional mitochondria are associated with chromosome errors, reduced fertilization, and blocked or compromised developmental. The thiol tripeptide glutathione (γ-glutamylcysteinylglycine, GSH) is the major intracellular redox buffer and represents a central element of the oocyte antioxidative system.

**Materials and Methods** In order to study the mitochondrial glutathione redox potential at different stages of oocyte maturation non-invasively, we have used a construct encoding for a mitochondrial signal sequence, the glutaredoxin-1 (Grx1), an enzyme that interacts with the GSH/GSSG system, and roGFP2 for specific live imaging of the mitochondrial redox potential (EmGSH). cDNA of Mito-Grx1-roGFP2 was cloned into a pSPo-vector and used for in vitro transcription. Polyadenylated mRNA was microinjected into GV stage oocytes from adult MF1 mice that were arrested in G2-stage by colistemide. The mitochondrial redox potential was analyzed at different stages of maturation after release from colistemide. Data were obtained by CLSM (Leica TCS SP2: Leica Microsystems) with sequential excitation of Mito-Grx1-roGFP2 by 405nm and 488 nm laser lines. Emission was recorded in the range of 500-530 nm. Cells were fully oxidized or reduced by exposure to 10mM H2O2 and 10mM DTT, respectively, and served as controls.

**Results and Discussion** Microinjected oocytes expressed the fusion protein in mitochondria. CLSM confirmed the enrichment of mitochondria in the vicinity of the oocyte spindle. The mitochondrial redox potential is related to the fraction of oxidized roGFP2 in the mitochondria. The amount of oxidized sensor was 43.5 ± 4.8 % (MV ± SE, n = 3) in maturation incompetent GV oocytes and 65.8 ± 3.4 % (MV ± SE, n = 6) and 63.3 ± 2.4 % (MV ± SE, n = 14) in GVBD and metaphase II (MII) oocytes, respectively. This corresponds to an EmGSH of –343.79 ± 2.60 mV for GV stage, and –331.26 ± 2.05 mV for GVBD and –333.45 ± 1.58 mV for MII oocytes. This is to our knowledge the first time the mitochondrial redox potential has been directly determined in living oo-
cytes. The method can now be used to assess dynamic alterations in mitochondrial redox potential in dependence of maturation conditions, age and cryopreservation of oocytes by different methods.

W-052 Oocyte Influence of oxidative stress on oocyte quality and protective role of melatonin as an antioxidant
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Poor oocyte quality is an important cause of female infertility. Reactive oxygen species (ROS) are produced within ovarian follicles, especially during ovulation process, and it is thought that increased ROS activity may be a cause of impaired oocyte maturation. PineaL hormone, melatonin, is detected in the human follicular fluid and melatonin is known as a potent free radical scavenger. This study was undertaken to evaluate the relationship between oxidative stress and poor oocyte quality and whether melatonin administration improves oocyte quality by protecting them from radical cytotoxicity.

Follicular fluid was sampled at oocyte retrieval during in vitro fertilization and embryo transfer (IVF-ET) program. Intrafollicular concentrations of 8-OHdG (8-hydroxy-2-deoxyguanosine) were determined as an oxidative stress marker, and Cu/Zn-superoxide dismutase (SOD), glutathione (GSH) and melatonin were determined as antioxidants. There was no correlation between SOD, GSH and follicular growth. However, melatonin concentrations increased depending on follicular growth. Although SOD and GSH did not show any correlation with 8-OHdG, melatonin showed a negative correlation with 8-OHdG. It seems that melatonin is the most important antioxidant in the follicle.

Oocytes recovered from immature ICR mice were incubated in medium with hydrogen peroxide (H₂O₂) and melatonin. After 12 hr incubation, oocytes with first polar body formation in oocytes were detected using a ROS dye (dichlorofluorescein). Reduced percentages of the oocyte with first polar body caused by H₂O₂ were recovered by melatonin addition. Fluorescence intensity caused by H₂O₂ was significantly decreased by melatonin treatment. H₂O₂ inhibits oocytes maturation but melatonin counteracted oxidative stress caused by H₂O₂.

Women who failed to become pregnant in the previous IVF-ET (fertilization rate < 50%) took 3 mg tablets of melatonin in the next IVF-ET, and fertilization rate and pregnancy rate were compared to the previous IVF-ET. The fertilization rate and pregnancy rate were improved by melatonin treatment. Intrafollicular melatonin concentrations were significantly increased and 8-OHdG levels were significantly decreased by melatonin treatment.

In conclusion, ROS have a toxic effect on oocyte maturation and melatonin may protect oocyte from ROS. Melatonin administration is likely to improve oocyte quality.

W-053 Ovulation induction Pulsatile application of FSH/LH in a PCO patient: A new approach to reduce or prevent severe ovarian hyperstimulation syndrome (OHSS)?
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Introduction Reducing the incidence of severe OHSS, particularly in PCO patients, is a challenge. Natural cycle or in vitro maturation (IVM) is a promising method to avoid OHSS but pregnancy rates are lower than with conventional IVF. Favorable experience with pulsatile application of GnRH in patients with primary hypothyroidic amenorrhea led to the idea of pulsatile application of FSH/LH in a PCO patient. The goal was to avoid over-recruitment of new follicles by applying small amounts of FSH/LH subcutaneously every 90 min.

Materials and Methods A 35-year-old patient with PCO (Rotterdam criteria) and st.p. bilateral salpingectomy was treated.
1. IVF/ICSI: Agonist long protocol: E 2: 6272 pg/ml on day before 5000 IU HCG. 46 eggs were retrieved. Two good quality embryos were transferred on day 3: 5 were cryopreserved. The endometrium was suboptimal and pregnancy was not achieved.
Kryo cycle: 5 embryos thawed, 1 for transfer. No pregnancy.
2. IVM: 20 eggs, 6× ICSI 2 Embryos with suboptimal quality were transferred.
3. IVF with pulsatile FSH/LH: After 2 cycles of oral contraception, triptoreline acetate 3.75 was given i.m. Two weeks later E 2 1 pg/ml and computer-assisted pump was applied which injected FSH and some days later FSH/LH every 90 minutes (16 times daily) a small amount of gonadotrophins subcutaneously: Day 0–5: 52.5 IU FSH/day. Sonography day 0: 10 antral follicles < 5 mm.
Day 6–7: 56 IU FSH/day plus 7.5 IU LH. Due to minimal response in E 2 levels, follicle growth and endometrial thickness on day 8 the dose of gonadotrophins was doubled and E 2 was given (3 × 2 mg orally and 3 × 2 mg vaginally).
Day 8–14: 105 IU FSH plus 15 IU LH/day.
Day 12 Sonography: right ovary: total of 15 follicles, largest follicle 13 mm Left ovary: 7 follicles, largest 13 mm. Endometrium 9 mm, 3 layer.
Day 14 largest follicle 17 mm, total 25–30 follicles. 6 p.m. stop hormone pump
Endometrium 11 mm, 3 layer, E 2: 3876 pg/ml (note: E, medication since day 8) 8 p.m. 10,000 HCG (36 hours prior to ovum pick up). Day 17: 27 eggs retrieved from all visible follicles. 12 × ICSI. Start progesterone 3 × 200 mg vaginally. Two good quality 8-cell embryos transferred on day 3, 1 blastocyst vitrified.
Hkt 40, 3 × luteal support 1500 HCG every 3rd day. Pregnancy testing negative

Conclusion Pulsatile application of gonadotrophins might be a new approach to prevent OHSS in PCO or even in all patients at risk of OHSS.

W-054 Ovulation induction Use of GnRH antagonist for ovarian stimulation with intrauterine insemination
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Artificial Insemination or Intratrate Inseminations is the older and simpler technique for treatment of infertile couples, now is used with ovary soft stimulation. Our paper assesses use or not use of GnRH agonist in hyperstimulation with pure FSHr cycles plus Artificial Insemination (AI). The objective was evaluate if GnRH antagonist used to not permit LH pulse and thus no permit ovulation in week end, without affect pregnancy rate. Retrospective case control study, 257 AI cycles, 202 (79.5%) without GnRH antagonist and 52 (20.5%) with GnRH antagonist. Both group were compared and have no statistic differences in estradiol serum level in HCG time, motile sperm counter (MSC), MCI (Mass Corporal Index) and Number of preovulatory follicles. Our results were pregnancy rate 9.6% per cycle in antagonist group and 15.8% without antagonist group (p: 0.46; n. s.). So, use of GnRH antagonist in AI cycles with soft hyperstimulation non affect the pregnancy rate.

W-055 Ovulation induction Successful pregnancy following iron chelation and ovulation induction for hypogonadotropic hypogonadism associated with beta thalassemia major: A case report and literature review
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Objective To describe infertility treatment and pregnancy outcome in a patient with transfusion-dependent beta-thalassemia ma-
major and hypogonadotrophic hypogonadism as well as results of a literature review on ovulation induction in beta thalassemia patients.

**Design** Case report and literature review.

**Materials and Methods** A 32 year old lady known to have Beta thalassemia major who presented with secondary amenorrhea due to hypogonadotrophic hypogonadism associated with iron overload. The patient was treated for two years with combined Desferal and Deferiprone followed by two years of Deferiprone iron chelation therapy to reduce the ferritin levels prior to ovulation induction. She underwent a step up ovulation induction protocol with Human Menopausal Gonadotropin and on serial ultrasound follow-up developed one dominant follicle. She received Human Chorionic Gonadotropin to trigger ovulation, followed by timed intercourse and oral progesterone for luteal support. We performed a “Pub Med” search of the English literature for ovulation induction in patients with Beta thalassemia major.

**Results** The patient conceived a singleton pregnancy on the first cycle of ovulation induction and there were no secondary complications of iron overload that developed or worsened during pregnancy. She underwent regular transfusions and was followed by a multi-disciplinary team. She delivered at term by emergency lower segment cesarean section for failure to progress in active labor. Both the patient and baby did well. From the literature review we found two articles on ovulation induction in thalassemic patients. One was a prospective trial in which Human Menopausal Gonadotropin was administered to 13 hypogonadal thalassemic women and in two out of three patients a dominant follicle developed. The second article looked at 19 thalassemic patients who underwent three cycles of induction of ovulation with Follicle-Stimulating Hormone.

**Conclusion** Iron chelation followed by ovulation induction with Human Menopausal Gonadotropin can result in successful conception in patients with hypogonadotrophic hypogonadism secondary to Beta thalassemia and iron overload.

**Funding** none.

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**W-056 Ovulation induction**

**The ART of gonadotrophin ovulation induction**

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Ovulation Induction (OI) with gonadotrophins has two main complications: Multiple birth and Ovarian Hyperstimulation Syndrome. In IVF, where a Birth Emphasising Successful Singleton at Term (BESST) [1] rate of 11% has been reported [2], single embryo transfer is becoming standard practice to reduce multiple birth. In OI or Controlled Ovulation Induction (COI) for OI/COH-IUI the Patient reported multiple birth. In OI or Controlled Ova-

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**W-058 Polycystic ovaries**

**The degree of cycle irregularity correlates with the grade of endocrine and metabolic disorders in PCOS patients**

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**Introduction** Polycystic ovarian syndrome (PCOS) is a clinically heterogeneous, endocrine disorder, which affects up to 4–10% of women in their reproductive age. A standardized definition is still difficult because of a huge variety of different phenotypes. The aim of this study was to evaluate possible correlations between the degree of cycle irregularity and the grade of endocrine and metabolic abnormalities.

**Methods** A cross-sectional study was carried out. Hyperandrogenic and/or hirsute women with regular menstrual cycles and polycystic ovaries in ultrasound (PCO: amenorr, n = 45), PCOS patients with oligo-menorrhoea (PCO oligo, n = 42) and PCO patients with amenorrhoea (PCO amenorr, n = 31) were recruited from the Department of Gynecological Endocrinology and Reproductive Medicine of the Women’s University Hospital Heidelberg (Heidelberg, Germany).

**Results** Normocyclic patients demonstrated metabolic parameters (BMI, Fasting Insulin,
HOMA-IR) significantly better than patients with oligoamenorrhea. Hormonal parameters (LH, FSH, FAI and testosterone) were significantly different between patients with different menstrual patterns and patient with regular cycles.

Conclusion Determining the degree of cycle irregularity as a simple clinical parameter might be a valuable instrument to estimate the degree of metabolic and endocrine disorders. Emphasis should be given to those parameters as a first step to characterize PCOS patients with a risk of endocrine and metabolic disorders leading to consequent detailed examination.

W-059 Polycystic ovaries
Effect of zone regimen and exercise on ovulation rate in women who are affected by polycystic ovarian syndrome

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Introduction Abdominal obesity in polycystic ovarian syndrome is major form of women obesity. This type of obesity more than another type can increase serum androgen and indirectly lead to infertility by suppressors of ovulation. In another hand, insulin resistance and hyper androgenism by direct relation, suppers the ovulation. According to important of non invasive and non drudge method in comprehensive treatment, we thought about weight loss and improve of insulin resistance. After study of many researches, we decided to use low carbohydrate regimen (zone) with regular calorie intake because of its low glucose intakes structure and weight loss effect. We thought that glucose intake maybe has effect on insulin resistance and hyper androgen improves and by less weight loss gain to more ovulation rate. The aim of this study was evaluating of ovulation rate after 3 month intervention (zone diet and exercise) in polycystic ovarian syndrome women.

Materials and Methods This is an interventional study and we used simple sampling method to collect data. We designed our study with 24 PCO women who were oligomenorrhea, overweight, obese and did not have any other hormonal disorder, then we advised zone diet with 1500 kcal/day and aerobic exercise (twice a week). After each week, patient completed 24 hour nutrition recall and after each month, we controlled ovulation by progesterone measurement in luteal phase (Gama Cutter machine). Data was analyzed by SPSS software version 14.

Results After first month of intervention we gain to 1/458 kg weight loss and 0% ovulation, in the second month, gain to 3/211 kg weight loss and 4/2% ovulation. At the end of study we gain to 5/046 kg weight loss and reached up to 29/2% of ovulation rate. The Wilcoxon test showed significant difference in ovulation rate at the third month compared to the start of the study (p = 0/004).

Conclusion Research showed that 7–10 kg weight loss improves ovulation and fertility in affected women but in our study we increase ovulation by less weight loss (5/046) during short time (3 month) (7–10 kg weight loss has been needed 6 more months). Our regimen is not restrict and low caloric diet therefore we can advise it for all fat people that are interested to weight loss. It can improve fertility in sub infertile or infertile women.

W-060 Polycystic ovaries
Effect of low carbohydrate regimen on menstrual interval in polycystic ovarian syndrome women

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Introduction Abdominal obesity in polycystic ovarian syndrome is major form of women obesity and this type of obesity more than another type can increase androgens. Researches showed that these complications have unhealthy effect on fertility and another system of women (endocrine, skin, cancers, …). A little loss of hip or waist circle can help patients to gain her healthy faster than general weight loss. We know that insulin resistance and hyper androgenism have direct relation together therefore we decided to use low carbohydrate regimen (zone) with regular calorie intake because of weight loss and low carbohydrate intakes of its structure. We think that carbohydrate intake maybe has effect on insulin resistance and hyper androgen improves. The aim of this study was evaluating of waist, hip and WHR rate after 3 months intervention (zone diet and exercise) in polycystic ovarian syndrome women.

Materials This is an interventional study and we used simple sampling method to collect data. We designed our study with 24 PCOs women who were oligomenorrhea, overweight, obese and did not have another hormonal disorder, then we advised zone diet with 1500 kcal/day (non restricted regimen) and aerobics exercise (twice a week). After each week, samples completed 24 hour nutrition recall and after each month waist and hip size was recorded and WHR rate was calculated. Data was analyzed using the SPSS software version 14.

Results After a 3-month-intervention, we showed significant decrees of waist (4/137cm) (P=0/001) and hip rate (3/608) (P=0/001) but WHR elevation (0/003) was not significant (P=0/228).

Average quality of general weight loss in first month was (1/45 kg), in second month was (3/221) and third month was (5/046). The Wilcoxon test showed significant difference in weight loss at the third month compared beginning of study (p = 0/001).

Conclusion Gynecology literature showed that 7–10 kg of weight loss can improve fertility and menstrual duration. There, use of restricted regimen in long time of 6 months was obligatory, but in our study we did not use none restricted in 3 months, we gained 5/046 kg weight loss and 54/2% menstrual regulation. It means that everybody can use of this regimen all day and they can achieve to weight loss and recovery of menstrual disorder. Patient by little weight loss, gain to better result therefore we can advise zone regimen to all affected women and none affected to prevention.

W-061 Polycystic ovaries
Effect of low carbohydrate regimen on waist, hip and WHR rate in polycystic ovarian syndrome women

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Introduction Abdominal obesity in polycystic ovarian syndrome is major form of women obesity and this type of obesity more than another type can increase androgens. Researches showed that these complications have unhealthy effect on fertility and another system of women (endocrine, skin, cancers, …). A little loss of hip or waist circle can help patients to gain her healthy faster than general weight loss. We know that insulin resistance and hyper androgenism have direct relation together therefore we decided to use low carbohydrate regimen (zone) with regular calorie intake because of weight loss and low carbohydrate intakes of its structure. We think that carbohydrate intake maybe has effect on insulin resistance and hyper androgen improves. The aim of this study was evaluating of waist, hip and WHR rate after 3 months intervention (zone diet and exercise) in polycystic ovarian syndrome women.

Materials This is an interventional study and we used simple sampling method to collect data. We designed our study with 24 PCOs women who were oligomenorrhea, overweight, obese and did not have another hormonal disorder, then we advised zone diet with 1500 kcal/day (non restricted regimen) and aerobics exercise (twice a week). After each week, samples completed 24 hour nutrition recall and after each month waist and hip size was recorded and WHR rate was calculated. Data was analyzed using the SPSS software version 14.

Results After a 3-month-intervention, we showed significant decrees of waist (4/137cm) (P=0/001) and hip rate (3/608) (P=0/001) but WHR elevation (0/003) was not significant (P=0/228).

Average quality of general weight loss in first month was (1/45 kg), in second month was (3/221) and third month was (5/046). The Wilcoxon test showed significant difference in weight loss at the third month compared beginning of study (p = 0/004).

Conclusion According to apple shape of obesity in poly cystic ovarian women and effect of this type on endocrinology and all system of patients, we think zone diet as a low
carbohydrate regimen can be advised by physician to polycystic ovarian women that they are interested to use a non restricted and daily regimen that can reduce weight loss and waist and hip circle can be reduced in short time without any major force on caloric intake.

**W-062 Polycystic ovaries**

**Clomiphene citrate and dexamethasone in treatment of polycystic ovary syndrome and infertility in Khoramabad**

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**Background** This study was to evaluate the effect of dexamethasone and clomiphene citrate in treatment of polycystic ovary syndrome (PCOS) and infertility.

**Materials and Methods** In this clinical trial study 120 infertile women with PCOS were randomly selected into two groups. Group 1 were treated with Clomiphene citrate in treatment of polycystic ovary syndrome (PCOS) and infertility. Group 2 were treated with placebo that was given from day 5 to day 14 of the cycle. The group of the study was ovulation and pregnancy.

**Results** There were no significant differences between groups in age, duration of infertility, BMI, menstrual pattern, hirsutism and serum DHEAS. The mean number of follicles on day 9 of the cycle and DEX 2 mg/day from day 5 to day 14 of the cycle. Group 1 were treated with Clomiphene citrate in treatment of polycystic ovary syndrome (PCOS) and infertility. Group 2 were treated with placebo that was given from day 5 to day 14 of the cycle. The group of the study was ovulation and pregnancy.

**W-064 Polycystic ovaries**

**Study for the differences of clinical features between the obese and the non-obese PCOS women**

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**Objective** The features of PCOS women in Asian countries are different from those in western countries, i.e. fewer cases with obesity and hirsutism. The purpose of this study was to analyze the clinical features of obese and non-obese PCOS women, and compare those with those in western countries.

**Methods** 76 PCOS women diagnosed by the Rotterdam criteria from April 2004 to December 2008 were divided into the obese (BMI ≥ 25, 29.3 ± 3.9, n = 18) and the non-obese group (BMI < 25, 20.5 ± 1.9, n = 58). HOMA index, the effectiveness of clomiphene citrate alone or with metformin, and the therapies conceived were compared between the 2 groups.

**Results** There were no differences between the 2 groups in terms of age, parity, and years of infertility. HOMA index significantly (p < 0.001) correlated with BMI, and was significantly (p < 0.001) higher in the obese group (2.96 ± 1.48) than the non-obese group (1.10 ± 0.57). The resistance rate to clomiphene therapy was higher, but not significant, in the obese group (n = 7, 38.9%) compared to the non-obese group (n = 12, 20.7%). There was no significant difference in the conception rate between the obese (n = 11, 61.1%) and the non-obese (n = 31, 53.4%) groups. However, the therapies conceived were different between the 2 groups, i.e. clomiphene alone therapy (n = 3, 37.2%), clomiphene with metformin therapy (n = 4, 36.4%), and gonadotropin therapy (n = 4, 36.4%) in the obese group, and timing therapy (n = 7, 22.6%), clomiphene alone therapy (n = 9, 29.0%), clomiphene with metformin therapy (n = 4, 12.9%), gonadotropin therapy (n = 5, 16.1%), and IVF-ET (n = 6, 19.4%) in the non-obese group. The combination therapy of metformin and clomiphene citrate was made in 11 of 18 obese and 14 of 58 non-obese PCOS women, and the conception rate showed a higher tendency in the obese group (n = 5, 45.5%) compared with the non-obese group (n = 4, 28.6%).

**Conclusion** Insulin resistance is rare in non-obese PCOS women, and 16 of 58 patients (27.6%) succeeded to conceive with the timing or clomiphene alone therapies. In contrast, obese PCOS women commonly have insulin resistance and need more intense infertility therapy compared with non-obese PCOS women. The combination therapy of metformin and clomiphene citrate is more useful in obese than non-obese PCOS women. The features of obese PCOS women are likely to be identical to those in western countries, while those of non-obese PCOS women are not.

**W-065 Polycystic ovaries**

**Comparison of menstrual pattern, clinical and hormonal effects of Acarbose with Metformin in polycystic ovarian syndrome**

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**Background** The aim of this study was to evaluate the effects of Acarbose and Metformin on clinical, hormonal and metabolic criteria in polycystic ovarian syndrome (PCOS) patients.

**Methods** This double blind randomized clinical trial was performed in PCOS with younger than 40 years old in Royan Institute. Using a computer – generated randomization, 40 patients were divided in two groups which treated by 300 mg/day acarbose or 1500 mg/day metformin. Body Mass Index (BMI), hormonal levels (SHBG, Testoster- one, FSH, LH, Prolactine, Cholesterol HDL and LDL) and menstrual pattern was evaluated before and after 3 months of treatment.

**Results** Final analysis in 24 patients in Acarbose group and 22 patients in Metfor-
min group manifested, in Metformin group the SHBG hormone significantly increased (21.24 ± 16.91, 28.73 ± 17.99; p = 0.001) after treatment. Meanwhile the FBS level, total cholesterol and LDL were significantly decreased. However the analysis in Acarbose group showed only the TSH hormone significantly decreased (1.97 ± 0.84, 1.50 ± 0.69; p = 0.012) after treatment. BMI was significantly decreased after treatment in both groups. Evaluation of pregnancy rate showed in Acarbose group from 20 persons 8 cases (40%) of pregnancy were occurred. But in Metformin group from 18 cases in 2 (11.1%) cases pregnancy were reported (p = 0.048). The rates of abortion in Acarbose group was 50% (4 cases) of pregnancy in Acarbose pregnancy in Acarbose group and 11.1% (2 cases) in Metformin group. However in Metformin group no abortion was reported.

Conclusion Metformin can significantly change some hormonal levels such as SHBG, FBS, total and LDL cholesterol more than Acarbose. The rate of pregnancy in Acarbose group was significantly higher than Metformin. However more abortion was reported in Acarbose group.

W-066 Polycystic ovaries
Phenotypic expression and BMI values in relation to LH levels in women with polycystic ovary syndrome

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It has been referred that normal-weight women with polycystic ovary syndrome (PCOS) present higher LH levels than those of overweight and obese women with the syndrome. However, it is not known if LH levels are influenced by the phenotypic expression of the syndrome. The aim of the present study was the comparison of LH levels between women with the classic PCOS phenotypes (1990 diagnostic criteria) and the newer PCOS phenotypes (2003 criteria) and the evaluations of body mass index (BMI) impact on LH levels. Nine hundred thirty six women with PCOS and 204 controls were studied. Women with PCOS were divided according to their phenotype in two groups. Group A included 729 women with classic PCOS, whereas group B included 207 women with ovulatory and without hyperandrogenemia PCOS. Group C included 204 healthy controls. Women of all groups were further divided according to their BMI, namely normal-weight (BMI < 25 Kg/m2) and overweight plus obese (BMI ≥ 25 Kg/m2). Group A: n = 381 and 348, respectively, group B: n = 118 and n = 89, respectively and group C: n = 123 and 81, respectively). Blood samples collected from all women between 8 and 11 AM, after an overnight fast. Serum LH, androstenedione, FSH, testosterone, ΔA, DHEA-S, 17α-OH-progesterone, LH/FSH, fasting insulin and glucose, insulin to insu-

W-067 Polycystic ovaries
Hyperandrogenemia and cycle length in mothers and non-mothers with PCOS: The LIPiCOS pilot-study

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Introduction PCOS-related symptoms like oligomenorrhea and hyperandrogenemia cover a broad spectrum of peculiarity. In this pilot-study we evaluate the correlation between the degree of oligomenorrhea and hyperandrogenemia with the history of pregnancy. Methods and Patients The LIPiCOS pilot-study invited women diagnosed with oligomenorrhea and infertility in 1991 to 2002 to a systematic follow-up visit after an average time of 12 years. Baseline testosterone, menstrual cycle length and history of infertility were compared and intra-individual change of cycle length, clinical signs of hyperandrogenemia, as well as serum testosterone, BMI and lifestyle changes were correlated with the occurrence of pregnancy in the preceding 12 years.

Baseline Results Cycle length of >35–89 days (n = 321; Ø infertility 3.6 years) was associated with longer duration of infertility than amenorrhea of >90 days (n = 82; Ø 1.3 years). However, cycle length did not significantly affect miscarriage rates (>35–89 days, 55 miscarriages; >90 days, 7 miscarriages, n.s.). Out of a total of 133 participants with total testosterone values available from the previous decade, TT was elevated in both oligo- and amenorrheic patients (n = 98, 0.6 ng/ml) vs n = 35, 1.14 ng/ml, n.s.). 70 patients without previous pregnancy (Ø age: 29.6 years) had elevated TT values (1.05 ng/ml), yet did not differ significantly from 23 women after miscarriage (Ø 33.7 years; TT 0.98 ng/ml) and 21 women after previous successful pregnancy (Ø 29.9 years; 0.96 ng/ml). Mean cycle length shortened over the years from 40 to 31 days (n = 58) but did not differ significantly between mothers (n = 45, age 40 years, BMI 22.1, 40 to 32 days) and patients without successful pregnancy (n = 13, 23 years, BMI 21.8, 45 to 30 days). 74.2% and 66.7% of mothers reported a shortening and a better cycle stability vs 63.6% and 53.8% of childless women. Akne and alopecia were more prevalent in the group of childless women (30.8%, 38.5% vs 11.1%, 15.6%).

Conclusion Although TT levels were elevated in these patients, high TT did not differ significantly with pregnancy outcome and cycle length. Clinical signs of hyperandrogenemia differed between participants having achieved pregnancy and childless oligomenorrheic women, with akne and alopecia being more prevalent in the latter.
levels were lower in mothers than in childless patients (0.45 vs 0.53 ng/dl). Regarding lifestyle interventions, mothers reported a healthier nutrition and a more regular everyday life compared to childless patients (85.7 vs 78.6%; 90.5 vs 68%). The standardized test meal revealed no impaired glucose tolerance or diabetes, but insulin secretion 60 min after glucose exposure was higher in childless women than in mothers (22.83 vs 18.05 IU/ml). Clinical hyperandrogenemia remained unchanged over the years.

Conclusion While age has already been shown to be associated with cycle length, the association of long-term intra-individual change of cycle length, insulin resistance, clinical hyperandrogenemia, and serum testosterone with the occurrence of pregnancy is new to be presented.

W-069 Polycystic ovaries
Role of fibroblast growth factor-9 (FGF9) and its receptor in regulating function of ovarian granulosa cells
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Insulin-like growth factor-1 (IGF1) is a naturally occurring hormone that has been implicated in the development of various cancers including ovarian cancer, but what genes are responsible for ovarian tumor development is unknown. Ovarian cancer is the most fatal of all gynecologic tumors, in part, because ovarian cancer has few symptoms early in its course, the majority of patients are diagnosed with advanced-stage disease with very low 5-year survival rates. Based on high-density microarray analysis comparing granulosa cell gene expression in IGF1 treated and control porcine granulosa cells, we discovered that fibroblast growth factor 9 (FGF9) mRNA is down-regulated and fibroblast growth factor receptor 2iiic (FGFR2iiic) mRNA is up-regulated in IGF1-treated granulosa cells. Ovarian vasculature increases during development of normal follicles as well as ovarian cancer/tumors, but molecular mechanisms that initiate ovarian angiogenesis are not well defined. The objective of the present study was to evaluate the hormonal regulation of FGF2riiic mRNA porcine granulosa cells, and evaluate the effect of FGF9, one of the ligands for FGF2riiic, on granulosa cell proliferation and differentiation. Granulosa cells were cultured for 48 h in 10% fetal calf serum and then treated with various treatments in serum-free medium for 24 h. Real-time RT-PCR was used to measure target genes. Neither FSH (30 ng/ml) nor estradiol (300 ng/ml) affected (p > 0.10) the IGF-I-induced suppression of THBS1 mRNA abundance. Specifically, IGF-1 (30 ng/ml) significantly down-regulated THBS1 mRNA in cultured porcine granulosa cells by 42–51%. In contrast, IGF1 and estradiol increased FGF2riiic mRNA abundance.

Cystic follicles had significantly greater FGF2riiic mRNA. Two-day treatment with 30 ng/ml of FGF9 increased (p < 0.01) basal and IGF1-induced basal production of severalfold, and stimulated (p < 0.05) IGF1-induced granulosa cell proliferation. We hypothesize that IGF1 may regulate THBS1 production by granulosa cells which in turn may contribute to changes in angiogenesis within the theca interna during normal follicular development as well as during formation of cystic follicles. IGF1 and FGF9 may concomitantly increase steroid production by granulosa cells. Thus, aberrant production of THBS1 and/or FGFR2 may lead to vascular dysfunction and ovarian disorders.

W-070 Pre-implantation embryo
Extensive alterations in the quantitative proteome profile during early embryonic development
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Early embryonic development is morphologically well characterized, but there is limited knowledge available about the underlying molecular processes. Several studies have addressed early bovine embryonic development on the transcriptome level, but to our best knowledge, no comprehensive approach on the proteome level has been performed so far. However, the generation of proteomic datasets is indispensable since a number of biochemical and physiological processes cannot be deduced from mRNA data, and early embryos contain a huge number of proteins originating from the oocyte protein pool. Due to strong similarities in estrous cycle (non-seasonal polyestrus), kinetics of early embryo development and singleton pregnancy, the bovine system reflects crucial parameters of the human reproductive system. Being interested in the dynamics of protein expression profiles during early embryogenesis, we here present a differential proteomic analysis comparing morulae and blastocysts. Protein lysates of embryos were analyzed using the ultrasensitive 2D DIGE Saturation approach, which facilitates quantitative 2D gel analysis from total protein amounts of < 0.5 µg. From 900 oocytes, 1221 proteins were approached on an Orbitrap XL mass spectrometer. 900 oocytes, 1221 proteins were identified. For 1045 proteins, existence on the protein level was experimentally proved for the first time in the bovine system.

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W-071 Pre-implantation genetic diagnosis
A case presentation of subfertile couple with t (4; 14) (q21; q32)
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Introduction An increased incidence of chromosomal translocations in couples who are habitual aborters has been reported. This is the first case presentation that presents translocation involving 4q and 14q with breakpoints q21; q32 can be regarded as a probably reason of recurrent pregnancy loss.

Case Presentation A couple was referred for cytogenetic examination due to idiopathic miscarriages. The proband proved to be a carrier of chromosomal translocation and her partner’s karyotype was found to be normal. The karyotype of the proband is 46, xx, t (4; 14) (q21; q32).

Conclusion This abnormal karyotype is reported for the first time as a probably reason of fertility problems in investigated couple. The risk of further miscarriage is high, but the risk of a progeny with abnormal karyotype is rather low, as the progeny would probably have lethal imbalances. Therefore, preimplantation genetic diagnosis is recommended.

W-072 Pre-implantation genetic diagnosis
Craniosynostosis, ptosis, hypodontia, prominent and everted lower lip, mental retardation: A second case of Mehta-Lewis-Patton syndrome
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Case Presentation An 11-year-old boy was referred to us for further investigation because he had dysmorphic features and was intellectually disabled. He was the first child of healthy, non-consanguineous parents. He was born with a low birth weight of 3300 g, length of 44 cm and a head circumference of 32 cm. There was a significant delay in developmental milestones. On clinical examination, he had a slender body with weight of 27 kg, his height was...
W-074 Pre-implantation genetic diagnosis

PGS by polar body biopsy – aneuploidy does not influence fertilization

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Introduction Preimplantation genetic screening (PGS) wants to improve artificial reproductive technologies (ART) by raising the rates of pregnancy, implantation and birth, decreasing the rates of abortion, multiple pregnancies and malformation and detecting patients which should retain from homozygous IVF but opt for oocyte donation. Led by a preclinical study with very high aneuploidy rates of failed to fertilize oocytes, also in young women, we conduct a cohort study at a University based IVF center with 100 patients without indications but offer it to all patients with a minimum amount of oocytes.

Methods After approval by the local ethics committee, we performed 104 PGs cycles between 2004 and 2008. PGS was offered to all patients with 8 or more oocytes, independent of age or other indications. It was performed by laser biopsy of the first polar body on day 0, fixation for FISH and hybridization with chromosomes 13, 16, 18, 21, 22. Zygote selection was carried out on day 1. In accordance with the German embryo protection law, and embryo transfer usually on day 2, with an intended elective double embryo transfer from proven “euploid” (for 5 chromosomes) oocytes. If no 2 euploid oocytes were available, possibly 1 or 2 non detected oocytes were transferred.

Results The average age of the women was 34 years (range 23–43 years). 1285 oocytes were retrieved, i.e. in average 12 per patient. Of these 1150 were mature (89%), of these 833 (93%) oocytes were transferred. Of the 893 biopsied oocytes 778 (87%) polar bodies could be fixed, of these we obtained genetic results for 621 (80%) oocytes. If no 2 euploid oocytes were available, possibly 1 or 2 non detected oocytes were transferred.

Conclusions The aneuploidy rate of human oocytes per patient is high even with a broad, non age related indication. Aneuploidy does not influence fertilization. The pregnancy rate does not significantly differ from the national average with ICSI (28%). In order to find the postulated benefits of PGS, indications should be made embryologically and clinically.

W-075 Reproductive endocrinology

Should AMH estimation replace the FSH estimation

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This study conducted in 60 patients. AMH estimation and day 2 estimation of FSH was done. It was found that FSH estimation was not much correlated with ovarian reserve and patients who were having FSH less than 10 but showing lower levels of AMH below 2 were poor responders and very less no of oocyte produced.

W-076 Reproductive endocrinology

Farid autotransfusion theory

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- 1-Eye tears Repeated Implantation failure (RF)
- 2-Repeated IVF failure
- Pelvic adhesion
- Asherman syndrome
- Cervical infertility
- POC
- Endometriosis
- Peripheral blood mononuclear cells: Repeated abortion
- Fibroid uterus – Asherman syndrome
- Endometriosis
- COC
- 5-urine: anew drug for treatment of endometriosis
- 6- FSH level
- PCO
- 7-AMH estimation
- 8-FSH level

Objective To predict ovarian reserve and clinical outcomes using basal FSH level for patients with bFSH ≥ 10IU/L.

Design Retrospective cohort study. 450 patients in group A with the bFSH level ≥ 10IU/L.

W-077 Reproductive endocrinology

The predictive value of basal FSH level for patients with the basal FSH level ≥ 10IU/L undergoing IVF/ICSI-ET

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Results The predictive value of basal FSH level for patients with the basal FSH level ≥ 10IU/L undergoing IVF/ICSI-ET was performed in 100 cycles (96%), resulting in 29 (29%) pregnancies, 20 deliveries and 9 abortions.

Conclusions The aneuploidy rate of human oocytes per patient is high even with a broad, non age related indication. Aneuploidy does not influence fertilization. The pregnancy rate does not significantly differ from the national average with ICSI (28%). In order to find the postulated benefits of PGS, indications should be made embryologically and clinically.
10–15 IU/L and one hundred and forty three patients in group B with the bFSH level ≥ 15 IU/L. Patients whose retrieved oocytes number < 5 were defined to poor responders.

**Intervention(s)** IVF/ICSI-ET using standard protocols.

**Main Outcome Measures** Oocytes number, the clinical pregnancy and early miscarriage rate.

**Results** Patients in group B was older and require more total gonadotropins and FSH dose initiated while had less AFC, aspirated follicles, oocytes retrieved, numbers of 2 PN and embryos transferred when compared with group A. No significant differences were noted between the two groups concerning the fertilization rate, pregnancy rate, early miscarriage rate and cycle cancellation rate. The ROC curves generated from the age, bFSH level, AFC were used to predict poor respond (the areas were: 0.685, 0.650, 0.812 respectively) and the pregnancy rate (the areas were: 0.65, 0.56, 0.67, respectively). According to bivariate correlation analysis, age, bFSH and AFC were negatively correlated to oocytes number (r = −0.370, −0.210, 0.511; p < 0.05) and pregnancy rate (r = −0.240, −0.097, 0.281; p < 0.05). After removing the effect of age, AFC, total FSH dose and FSH dose initiated, the partial correlation analysis showed that FSH level was also negatively correlated to the number of retrieved oocytes, but not to the clinical pregnancy rate. Number of retrieved Oocytes (Y) could be well predicted in a multiple regression analysis prediction model that included AFC, age and bFSH rather than the pregnancy rate. Instead, age and AFC may be better predicted by the combination of the AFC, age and bFSH rather than single predictors. Patients whose bFSH level ≥ 15mIU/ml got lower pregnancy rates than those with 10IU/L – 15 IU bFSH level. This may be caused by the reduced number of oocytes retrieved, not by the poor quality of oocytes. In other words, patients with high basal FSH level could still receive IVF/ICSI-ET.

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**W-078 Reproductive endocrinology**

**Luteal blood flow and luteal function**


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**Background** Blood flow in the corpus luteum (CL) is important for the development of the CL and maintenance of luteal function. We recently reported changes in blood flow in the human CL throughout the luteal phase and luteal blood flow was significantly lower in women with luteal phase defect (LFD) than in women with normal luteal function. This study was undertaken to investigate whether luteal function can be improved by increasing CL blood flow in women with luteal phase defect. We focused on vitamin E and a potential nitric oxide (NO) donor, L-arginine, to increase luteal blood flow.

**Methods** Informed consent was obtained from all the patients in this study. A total of 66 women who had LFD and high blood flow impedance of the CL were recruited into this study. When serum progesterone (P) concentrations were < 10 ng/ml during the mid-luteal phase, the patient was diagnosed as having a LFD. Blood flow impedance in the CL was measured by transvaginal colour pulsed Dopplerultrasorography and was expressed as a resistance index (RI). Receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off value of the CL-RI (≥ 0.51) providing the best values of sensitivity and specificity for determination of normal luteal function and LFD. The patients with both LFD (serum P concentrations < 10 ng/ml during mid-luteal phase) and high CL-RI (≥ 0.51) were given Vitamin E (600 mg/day, n = 18), L-arginine (6 g/day, n = 14) as a potential nitric oxide donor, melatonin (3 mg/day, n = 13) as an antioxidant, or HCG (2,000 IU/day, n = 10) during the subsequent menstrual cycle. As controls, 11 patients received no medication. To evaluate the effect of those treatments, serum-P-concentrations and CL-RI were measured during the mid-luteal phase.

**Results** In the control group (n = 11), only one patient (9%) improved in CL-RI and 2 patients (18%) improved in serum P. Vitamin E improved CL-RI in 15 patients (83%) and improved serum P in 12 patients (67%). L-arginine improved CL-RI in all the patients (100%) and improved serum P in 10 patients (71%). HCG improved CL-RI in all the patients (100%) and improved serum P in 9 patients (90%). Melatonin had no significant effect.

**Conclusion** Vitamin E or L-arginine treatment improved luteal function by decreasing CL blood flow impedance. CL blood flow is a critical factor for luteal function.

**W-079 Reproductive endocrinology**

**The effect of growth factors on IVF/ICSI outcome in obese patients**


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**Introduction** In women, obesity is often associated with menstrual disorders, infertility and disappointing results in medically assisted procreation. Its mode of action on fertility and insulin is complex but is primarily responsible for endocrine disrupters on insulin, insulin-like growth factor-1 (IGF1).

The aim of our study is to determine changes in these growth factors in patient’s infertile obese or overweight patients receiving in vitro fertilization (IVF) and seek correlations between hormonal disturbances and the biological parameters of IVF.

**Materials and Methods** Our work focuses on 91 patients recruited from the Unit of Reproductive Medicine of Sousse, consulting for infertility and who would benefit from assisted conception techniques.

This population was divided into three groups according to BMI. A control group (normal weight), an overweight and obese group. 2 types of sampling were conducted, one blood sample and a sampling of follicular fluid (FF) made the day of follicle aspiration. The hormone assays of IGF1, IGFBP3 and insulin were performed by immune radiometric method.

**Results and Discussion** The rate of IGF1 was significantly lower in obese patients compared to controls in both the FF and in the serum. This finding was confirmed by the existence of a negative correlation between rates of IGF1, serum follicle, and BMI in all patients. There is also a positive correlation between the rate of IGF1 and the fertilization rate (p = 0.04). Pregnant patients have higher rates of IGF1 higher than non-pregnant patients. The average of insulin levels among groups of obese patients and overweight are higher than patients of normal weight, more insulin levels of pregnant patients were lower than non-pregnant patients.

**Conclusions** The results of the present study identified that obesity is accompanied by a disturbance in the rate of IGF1 (serum and follicular) and it alters the rate of fertilization. The benefit of weight loss on fertility in obese patients is certain particular patients’ candidates for IVF.
W-080 Reproductive endocrinology
Reproductive function of women after medical abortion
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Objective The aim of the study was to define the effect of medical abortion on reproductive function in women.

Methods 300 women were investigated after medical abortion (600 mg of mifepristone + 400 mg of misoprostol per os) for 2–6 weeks of pregnancy. 50 patients’ hormonal status was investigated in order to identify the hormonal changes in hypophysis-ovarian system. For this purpose the concentration of gonadotropins, estradiol and progesterone in blood serum was defined. The changes of concentration of progesterone (defined before medical abortion) and estradiol were observed in dynamics during the period of 30–35 days.

Results The analysis of the results show that the ovulation in the post abortion menstrual cycle was restored in 78.5% of the patients. On average approximate days of ovulation made 13.6 ± 1.3 and in the same days the average thickness of the endometrium was 10.4 ± 0.7 mm. Assessment of the hormonal status proved that concentration of progesterone reduced up to 2.5 times within 48 hours after the intake of mifepristone, which enabled us to suppose that termination of pregnancy during medical abortion takes place particularly within the 48 hours after the intake of antiprogestin.

Conclusions Medical abortion has a sparing effect on reproductive system of a woman and in most of the patients the ovulation in post-abortion menstrual cycle is restored, which proves that medical abortion is characterized with minimal risk of infertility caused by anovulation.

W-081 Reproductive endocrinology
Localization of Hormone-sensitive lipase (HSL) in the rat ovary and reproductive tract during the estrous cycle
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Hormone-sensitive lipase (HSL) is a key regulator of cholesterol esters metabolism in steroidogenic tissues. The aim of this study was to determine HSL localization in rat female reproductive organs during the ovary cycle by immunohistochemical methods. HSL was located in the ovarian epithelium. The granulosa cells and oocytes of primordial follicles were immunonegative. In mature follicles, HSL was found in oocytes, theca and granulosa cells. However, HSL expression in theca cells and oocytes decreased during follicular atresia. Luteal cells showed HSL staining in cytoplasm during preovulatory and estrous, in nucleus during metestrus, and cytoplasm and nucleus during diestrus. In the tubaric ampulla, HSL was located in the epithelial cells nuclei in and the cylia during proestrus and estrus, but mainly in nucleus during metestrus and diestrus. In the isthmus, cells showed HSL immunolabeling in the nucleus and cilia during proestrus, but only in the cilia during estrus, metestrus and diestrus. In the uterus, HSL was found in the epithelial cells nuclei. In the vagina, the strata basalis and the first layer of the spinous strata cells showed positive HSL staining mainly restricted to the cytoplasm during proestrus and diestrus, whereas the staining disappeared of the cells of the strata basalis and appeared in the nucleus of the cells of the strata spinous during estrus and metestrus. Western blotting analysis revealed HSL-immunoreactive bands at 84, 67, 54 and 43 kDa in rat female reproductive organs.

Conclusions The expression and localization of HSL in the rat female reproductive organs change during the ovarian cycle in a stage-specific and cell-dependent manner. The presence of HSL in theca and granulosa cells of normal rat ovaries suggests that cholesterol ester activity of this protein may play a key role in steroidogenesis through the supply of free cholesterol for steroid hormone synthesis. This protein is also involved in oogenesis, as its presence in oocytes suggests, supporting the growing body of evidence that HSL may have important roles in the reproductive system and in gamete maturation in female rats. And finally, HSL labeling in the nucleus of epithelial and germ cells suggest an, as yet, unknown function for this enzyme, probably related to cell proliferation.

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W-082 Reproductive endocrinology
Expression of FSH receptor mRNA in granulosa cells is low in women with AA** genotype
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The follicle stimulating hormone (FSH) plays a pivotal role in ovarian folliculogenesis. FSH action is regulated by altering serum FSH levels and by controlling number of competent receptors present at the surface of granulosa cells surrounding the oocyte. The ovarian response to FSH and the level of expression of its receptor is shown to be variable in different women undergoing in-vitro fertilization (IVF) treatment. Associations of the single nucleotide polymorphisms in the FSH receptor gene with ovarian response have been studied extensively. The aim of this study was to examine the association of the polymorphism in the promoter region with the level of FSH receptor gene expression and to examine their role in ovarian response in women undergoing IVF treatment.

In the present study, 84 infertile normogonadotropic ovulatory women undergoing controlled ovarian stimulation during IVF program were recruited retrospectively. RNA was extracted from granulosa cells and was reverse transcribed. The relative expression of FSH receptor mRNA was analyzed using quantitative polymerase chain reaction (PCR). Genomic DNA was extracted from blood obtained from these women and used for PCR and restriction fragment length polymorphism (RFLP) analysis to determine the genotype at –29 position of 5’ untranslated region (5’ UTR) of FSH receptor gene. Statistical analysis was carried out using SPSS software. Results revealed significantly lower expression of FSH receptor gene in women with AA** genotype suggesting that the polymorphism at –29 position plays an important role in FSH receptor gene expression. Also women with AA genotype required higher amount of FSH for ovulation induction implicating their resistance to FSH treatment. Thus our findings strongly indicate that polymorphism at –29 position of 5’ UTR of FSH receptor gene is associated with lower expression of FSH receptor in granulosa cells and these women demonstrate poor ovarian response. Thus screening of FSH receptor genotype may be useful as a biomarker for determining poor ovarian response in women undergoing IVF treatment.
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W-083 Reproductive endocrinology
a2V-ATPase expression on neutrophils in pregnant women with reproductive disorder

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Purpose a2V-ATPase (a2V) is found in immune cells and has cytokine-like effects that are pro-inflammatory and immune regulatory in nature without being detrimental to cells or tissue. a2V-ATPase is the a2 isoform of vacuolar ATPase and is expressed in human trophoblast cells. There are several lines of evidence suggesting the importance of a2V-ATPase in human pregnancy.

Previous findings have shown that a2V is differentially expressed in peripheral blood lymphocytes of pregnant versus non pregnant women who had a history of recurrent spontaneous abortions. This has led us to hypothesize that differences in surface expression of a2V may be controlled by physiological changes that occur throughout pregnancy. We will focus on peripheral blood cells, where we have previously reported differences between pregnant and non-pregnant women and will extend this to neutrophils.

The aim of this study was to characterize the a2V expression in neutrophils from pregnant versus non pregnant women who had a history of recurrent spontaneous abortions.

Materials and Methods Whole blood was obtained from the study groups. For staining for a2V, 100 µL of whole blood was incubated with anti-a2V-AF647 mAb (2C1) and anti-CD66b for neutrophils or isotype control for 45min, followed by lysing and fixation. Results were analyzed by flow cytometry. The gate was set on the neutrophils which were subsequently analyzed for % expression of a2V. For each sample, 104 peripheral blood neutrophils were analyzed. Serum from study groups were also collected and tested for estradiol and progesteron, which were subsequently analyzed for correlation with a2V.

Results There was a significant difference in the expression of a2V on neutrophils of non-pregnant women versus pregnant women in the 1st trimester (10.5 ± 8.2% vs 27.8 ± 16.2%, respectively, p = 0.0001), and pregnant women in the 2nd trimester (10.5 ± 8.2% vs 27.8 ± 24.1%, respectively, p = 0.0007). There was no statistically significant correlation between a2V expression and hormone levels.

Conclusions These results indicate that pregnancy may influence the a2V-ATPase expression in neutrophils.

W-084 Reproductive endocrinology
C60 Fullerenes may be used in very low doses for applications in human ovaries

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Introduction In the last years various nanomaterials have raised interest in numerous potential biomedical applications. One of them, the C60 Fullerenes, are composed of 60 carbon atoms in the shape of a football and are considered for imaging probes, antioxidants or drug carriers. However, one difficulty in studying the activities of fullerenes in biological systems is that they are poorly soluble in aqueous media. Therefore, organic solvents are routinely used for solubilization of C60 Fullerenes in water. The aim of this project was to determine if fullerenes may also be used in ovarian applications by examining the production of interleukin 6 (IL-6) and Ilbeta (IL-1beta) in cultured granulosa cells.

Methods Granulosa cells were obtained during oocyte retrieval from women undergoing in vitro fertilization. After purification cells were seeded in 24-well plates and cultured with increasing amounts (0.025, 0.1 and 0.4 mg/ml) of C60 Fullerenes prepared by two different methods.

• Culture wells were coated with a fullerene-suspension in methanol following solvent evaporation and cell seeding.
• Fullerenes were dissolved in toluene and transferred into water by sonication. These water-soluble fullerenes were then added to the cultured cells. After four days incubation IL-6 and IL-1beta were quantified in the culture supernatant by enzyme-linked immunosorbent assay (ELISA) and compared between fullerene-treated cells and untreated control cells.

Results We found in the ELISA assays that granulosa cells secreted much higher amounts of IL-6 into the culture supernatant than of IL-1beta. Cells cultured with 0.025 and 0.1 mg/ml C60 Fullerenes prepared by the methanol method produced the same amounts of IL-6 and IL-1beta like the untreated control cells. Cell treatment with 0.4 mg/ml fullerenes resulted in significant 5-fold and 4-fold increases in the IL-6 and the IL-1beta concentrations. However, granulosa cells incubated with fullerenes prepared by the toluene method already showed with the addition of 0.1 mg/ml nanomaterial a significant IL-6 increase of 39 % and a significant IL-1beta increase of 85 % in comparison to untreated control cells.

Conclusion Our results show that C60 Fullerenes do not influence the IL-6 and IL-1beta production of granulosa cells at the very low dose of 0.025 mg/ml and may be used for diverse applications in human ovaries. This project was supported by the University Hospital Aachen, Germany.

W-085 Reproductive genetics
Analysis of imprinting genes in offspring conceived by assisted reproductive technologies with gene microarray

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Background Recently, concerns about imprinting disorders linked to assisted reproductive technologies (ART) have been increasing. Abruptant DNA methylation of imprinted in many genes are found in the majority of children with imprinting disorders conceived by ART. For future studies analyzing specific genes separately, we did gene microarray analysis in our pilot study, for primarily screening differential some imprinted genes with possible abnormal DNA methylation in ART-conceived babies.

Methods A total of 4 babies (3 of them were conceived after IVF, ICSI, frozen-thawed embryo transfer (FET), respectively; 1 baby was conceived after natural pregnancy) were included. Genomic DNA of each infant were isolated from Ccord-blood samples (8–10 mL), then fragmented in size about 500bp using ultrasound followed by MCdIP. Scanning was performed with the Agilent DNA microarray scanner (part number G2505B) using settings recommended by Agilent Technologies. Baby conceived naturally was regarded as a control, and the other three babies were study objects. Differential DNA methylation genes were screened between them.

Results Babies conceived naturally and conceived by FET was born with term delivery and normal birth weight (BW). The other two babies were born with premature delivery and low BW. 20 differential DNA methylation genes were found among the four samples. In the promoter region, five genes showed a methylated status compared with the control case, and 15 genes showed an unmethylated status. These genes included transcription factor related-genes, metabolic enzyme-related genes, tumor related-genes, immune-related genes, heat shock-related genes, zinc finger protein-related genes, cytoskeletal protein-related genes, extracellular matrix genes, and cell adhesion molecules, growth factors, immune-related genes, and so on.

Conclusions A number of differentially methylated genes are screened out by the technology of High-throughput DNA methylation of the gene array. The results can help to analyze imprinting genes and imprinting disorders. But further research is needed to verify our results.

Funding This study was supported by the National Basic Research Program of China (973 program) (2007CB94840104).
W-086 Reproductive genetics
Different polymorphic variants of the karyotypes of patients in IVF programs

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The influence of the heterochromatin regions of chromosomes in the manifestation of different clinical features remains an unclear question.

According to many authors, heterochromatin variation in couples with reproductive impairment and/or burdened obstetric history were of a non-random nature. We conducted a retrospective analysis of the frequency of different polymorphic variants of the karyotypes of patients in IVF and ET programs. The distribution in patients with primary and secondary infertility, as well as communication options karyotypes quality embryos and the onset of pregnancy.

The cytogenetic study of couples was carried out, not only to exclude chromosomal pathology, but also for the recording and analysis of polymorphism in the heterochromatin regions of individual chromosomes and their occurrence and possible relationship to the results of IVF and ET programs.

We have not noticed significant differences in the frequency of variants of rules in the karyotype in primary and secondary infertility (14% vs 14.7%).

However, we found a significant difference in the quality of embryos in primary and secondary infertility in patients with a normal karyotype (58.3% vs 41.7% and 78.6% vs 21.4%). Patients with variants in the karyotype did not have significant differences in embryos quality.

However, definitive data on the effect of polymorphic variants of the karyotype on the quality of embryos, pregnancy, and their frequency in the primary and secondary sterility haven’t been noticed, although there was an increased rate of pregnancy in the secondary infertility and normal karyotype, compared to primary infertility and variations in karyotype (41.4% vs 12.5%).

We recommend further accumulation of data, through a multicenter study to examine polymorphism in families with infertility.

W-087 Reproductive genetics
Mannose-binding lectin-2 (MBL2) gene polymorphisms and their impact on pregnancy outcome

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Introduction Mannose-binding lectin (MBL) is a plasma protein, which, upon binding to microbial carbohydrate structures, activates the complement system. A genetically determined deficiency of plasma MBL has been shown to predispose to a variety of infectious and inflammatory diseases. Recently, low MBL plasma levels have been reported to have a negative impact on pregnancy outcomes in women with idiopathic recurrent pregnancy loss (RPL). The purpose of this study was to investigate the association of MBL2 genotypes in RPL and normal pregnancy.

Materials and Methods 78 women with a history of > 2 consecutive, idiopathic miscarriages were analyzed for genetic polymorphisms in the MBL2 gene, which largely determine serum MBL levels. 56 of the RPL patients suffered from late RPL (LRPL) > 16 week of gestation. LRPL patients were further subdivided into patients with clinical signs of infection (n = 22; premature rupture of membranes, chorioamnionitis) and idiopathic LRPL (n = 34). 123 healthy pregnant women (14.–38. week of gestation, no miscarriages) served as controls. Genotypes were determined by DNA sequence analysis and categorized into high- (HYA/HYA, LYA/LYA, HYA/LYA, LYA/LXA, LYA/LYA), intermediate- (HYA/0, LYA/0, LXA/LYA, LXA/LXA), and low-producing genotypes (0/0, 0/LXA) associated with high, intermediate and low MBL plasma levels, respectively.

Results Our results show no significant difference in the frequency of the high-, intermediate-, and low-producing MBL2 genotypes between RPL patients and pregnant controls. Also, LRPL-patients did not differ from pregnant controls. High genotypes were found in 43.6% RPL patients, 44.6% LRPL patients and 46.3% pregnant (n.s.). Intermediate genotypes were present in 37.2% RPL patients, 33.9% LRPL patients and 34.9% controls (n.s.). Low genotypes were detected in 19.2% RPL patients, 18.7% LRPL patients and 18.7% pregnant (n.s.).

We also did not detect any differences in the frequency of the various MBL2 genotypes for the subgroups of LRPL patients with infectious signs or idiopathic. Compared with literature, our analyses demonstrated a higher prevalence of the low genotypes in pregnant controls (18.7%) than has been reported for healthy European females (6–12%).

Conclusion We failed to confirm the reported increased prevalence of low-produc-

W-088 Reproductive genetics
MBTHF gene epigenetic affects pregnancy outcome

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Introduction It has been demonstrated that methyltetrahydrofolate reductase (MTHFR) is expressed in human oocytes and preimplantation embryos. Insufficient availability of methyl groups for DNA, protein and lipid methylation could impair proliferation and differentiation of the granulosa layer, thus inhibiting follicular performance and as well as steroidogenesis. It is suggested that aberrant methylation, including due to the culture environment leads to loss of imprinting and/or changes in gene expression. Our hypothesis in this study is that mutations in MTHFR could compromise oocytes and embryo development, which could result in decreased ongoing pregnancy outcome.

Materials and Methods A prospective study of 130 women, 105 with infertility and 25 without. At 54 patients were performed intrauterine insemination (IUI) and 44 intravitro fertilization/embryo transfer (IVF/ET). All women were tested for C677T mutation (C/T and T/T variants) of MTHFR by polymerase chain reaction. Outcome measures were the type of influence of MTHFR mutation on fertility, ovarian response, ovum quality, embryo-development and short-term pregnancy outcome. Descriptive statistical analyses by Crosstabulation, Chi²-test analysis and Fisher’s exact test were performed.

Results We found out in patients with infertility and mutation of MTHFR (60 females) significantly higher frequency of MTHFR mutations among non-pregnant (44/60-81.7%) compared with conceived (11/60; 18.3%; p = 0.00). This trend was similar in the group of IVF/ET (21.7% pregnant vs 78.3% not pregnant, among patient with MTHFR; p = 0.03) and in IUI-group (24.2% pregnant vs 75.8% not pregnant; p = 0.04). Additional analyses showed T/T with insignificant results (p = 0.81), whereas C/T was found in significantly higher number of cases (82.4%) with no pregnancy (p = 0.007). In patients without infertility did not observed a such significant difference (p = 0.11). Significantly greater incidence of MTHFR mutation has been observed in low responders of IVF/ET group (85.7%; p = 0.05) as the C/T was significantly higher (p = 0.05). T/T in most cases had no significant impact, but in IVF/ET was found that at all patient with this type of mutation was picked up immature eggs (100%, p = 0.05).

Conclusions The received results suggest that expression of MTHFR mutation and par-
**W-089 Reproductive genetics**

The potential use of array-based comparative genomic hybridization for cytotgenetic analysis of spontaneously expelled miscarriages

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**Introduction**

Cytogenetic analysis of miscarriages provides important information regarding the future pregnancy for couples with recurrent spontaneous abortions (RSA). Although G-banding method is used for the analysis, a scheduled dilation and curettage (D&C) is generally necessary to obtain cultivable samples. On the other hand, non-surgical managements have been recently performed for selective miscarriage cases instead of D&C. In the present study, we tested the potential of array-based comparative genomic hybridization (array-CGH) for cytogenetic analysis of spontaneously expelled miscarriage samples, which are usually inappropriate for G-banding analysis due to poor culture quality.

**Materials and Methods**

We analyzed 21 miscarriages expelled spontaneously, following the protocols approved by the institutional review board. Genomic DNA was extracted from villous tissue and analyzed by the targeted array-CGH including 550/660 BAC clones (100–200 kbp/clone) covering every subtelomeric region. In order to rule out maternal contamination, STR (short tandem repeat) analysis was performed for the cases with normal female results. For the case with partial aneuploidy, the tiling BAC array was used to evaluate the exact region of the genomic imbalances.

**Results**

The targeted array-CGH was successful in all 21 cases. 12 cases had unbalanced gains of copy number and three cases had unbalanced losses. The majority of these cases were estimated as autosomal aneuploidies. Only one case showed partial monosomy (1p36.33-32), and proved to be 7Mb deletion by the tiling array-CGH. Maternal contamination was detected in three of four cases with normal female results.

**Conclusions**

Array CGH is a powerful tool for cytogenetic analysis of spontaneously expelled miscarriage samples. It also allows us to detect submicroscopic abnor-mality, which is difficult to detect by standard G-banding method. Since it is often difficult to distinguish choriionic villi from maternal tissues due to extensive degeneration in the samples brought to the hospital, ruling out maternal contamination is necessary for the cases with normal female results. The present diagnostic system makes a great contribution to the management of the patients with RSA, who desire non-surgical management due to great apprehension over having repeated operations.

**Financial support**

This work was supported mainly by a Grant for Child Health and Development from the Ministry of Health, Labor and Welfare (20C-1).

**W-090 Reproductive genetics**

Human homolog piwi-like 1, PIWIL1 binds to rasiRNAs which seems to silence the expression of repeat sequences in early stage of spermatogenesis

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**Introduction**

Male spermatogenesis is one of dynamic process which endures morpho-genetical and genetic changes associated with expressions of developmental stage dependent genes. PIW1 family is one of candidate, assuming that it controls germ cell division and small RNAs during spermatogenesis. In human, the role of PIW1 family is still elusive, we here strive to verify function of human homolog PIW1 (HIWI) during male spermatogenesis.

**Materials and Methods**

Testis extracts are collected from Sertoli only syndrome patient and normal spermatogenesis male which are used for RT-PCR, Western blotting and RNA immunoprecipitation. To analyze sequences of small RNAs, we perform sequencing using on Genome Sequencer GS-FLX instrument (454, Roche). Total reads 31,800 from Sertoli cell only syndrome and 54,161 from normal spermatogenesis are mapped to the human reference genome, NCBI build 36 and miBase v12.0. To analyze types of rasiRNA, reads were identified from the genomic sequences in RepeatMasker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) that uses a cross-match program to perform perfect sequence alignments, and from the Repbase database.

**Results and Discussion**

HIWI is exclusively expressed in male germ cells not found in other somatic cells in testis. Moreover, the localization signal of HIWI is strongly expressed in cytoplasm and weakly positioned in nucleolar region of spermatocyte. From these results, HIWI seems to be related with early stage of spermatogenesis and may play a role with cytoplasm of spermatocyte. To confirm our hypothesis, we examine the binding activity of small RNAs with RNA immunoprecipitation using testis extracts. 24–26 nt sized small RNAs bind to HIWI protein and other sizes are not identified to relate with HIWI. Most of 24–26 nt is derived from repeat sequence associating rasiRNAs which is matched with Alu, LINE and HERVs To clarify our result, we compare each small RNAs derived from only somatic cells and normal spermatogenesis in testis which show that around 57% (10,774) of sequences from somatic cells matched with miRcoRNAs. Conversely, only 2% (749) sequences are matched with microRNAs in normal spermatogenesis. Moreover, over 50% of small RNAs are matched with rasiRNAs in normal spermatogenesis meant that PIWI play a significant role control expression of rasiRNAs. Taken together, PIW1 binds to rasiRNAs which seems to silence the expression of repeat sequences in early stage of spermatogenesis.

**W-091 Sperm biology**

Theophylline and capacitation of buffalo spermatozoa

El-Amrawi G.A.1, Elbawab E.2,3., Hussein F.M.2


Theophylline was added to buffalo semen (0.5 mg/ml) to study its effect on the time required for sperm capacitation. 1 ml of semen was introduced into rabbit uterus in estrus and recovered after intervals of 60, 90 and 105 minutes and stained by silver nitrate stain. The other portion of the semen was incubated at 37°C for 105 minutes. The results indicated that the swelling of the acrosome was observed at 60 minutes after intrauterine deposition. The peeling of the acrosome started 90 minutes after intrauterine incubation of semen sample. Equatorial segments were clearly defined 105 minutes after incubation. No morphological changes were found in the sperm cells incubated in vitro. Results of the present study indicated that theophylline accelerated the capacitation process.

**W-092 Sperm biology**

Effect of theophylline on capacitation of buffalo spermatozoa

El-Amrawi G.A., Elbawab E.2,3., Hussein F.M.2


Theophylline was added to buffalo semen (0.5 mg/ml) to study its effect on the time the required for sperm capacitation. 1 ml of the semen was introduce into rabbit uterus in estrus and recovered after intervals of 60, 90 and 105 minutes and stained by silver nitrate stain. The other portion of semen was incubated at 37°C for 105 minutes. The results indicated that the swelling of the acrosome was observed at 60 minutes after intrauterine deposition. The peeling of the acrosome started 90 minutes after intrauterine incubation of semen sample. Equatorial segments were clearly defined 105 minutes after incubation. No morphological changes were found in sperm cells incubated in vitro. Results of the present study indicated that theophylline accelerated the capacitation process.
W-093 Sperm biology
A study to determine the relationship between increased semen viscosity and male genital tract infections

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Introduction A routine semen analysis remains the cornerstone of an andrological evaluation. Several semen variables are evaluated but viscosity is rarely quantified. A trend has emerged whereby patients attending the Reproductive Biology Unit at Tygerberg Hospital in South Africa have shown to be presenting samples with increased viscosity. The reasons behind this developing phenomenon have yet to be determined. A possible explanation could lie in the increase of male patients presenting with Leukocytospermia. Male genital tract infections (MAGI) is reflected by increased numbers of white blood cells (WBC) in semen and is associated with impairment in semen quality.

Outcomes The main outcome of this study is to investigate the relationship between semen samples presenting with hyperviscosity and male genital tract infections. Studies into the cause behind hyperviscosity and its relationship to altered sperm function and dysfunction of the male accessory sex glands would provide vital insight into a condition that has received little attention.

Hypothesis 1. The condition of Leukocytospermia, an indicator of the presence of a male genital tract infection, is associated with increased seminal viscosity and 2. The consequential hypofunction of the seminal vesicles and prostate as a result of MAGI, results in semen hyperviscosity.

Specific Aims The aim is to investigate the relationship between increased viscosity of semen samples and the presence of male genital tract infections. Investigations will include the following: detection of the presence of leukocytes and the assessment of the prostate and secretory vesicle functioning. All investigations undertaken will include a detailed analysis of the semen parameters including morphology, concentration, viscosity status and motility.

Location and Infrastructure All experimental procedures and research will be performed in the Andrology Laboratory at the Unit for Reproductive Biology at Tygerberg Hospital, South Africa.

Funding The main source of funding for this research project was provided by the Harry Crossley Foundation.

W-094 Sperm biology
Sperm DNA fragmentation and maturity on the day of ART and impact on cycle outcome

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Objective To evaluate sperm DNA fragmentation and DNA maturation in samples used for ART as indicators of DNA damage in the embryo quality, index of fertilization, pregnancy outcome and implantation.

Design Retrospective Study.

Patients 30 infertile men participating in the IVF/ICSI at the IIECH, Monterrey.

Intervention Halosperm Kit and Aniline Blue Staining.

Main Outcome Measure(s) Degree of sperm DNA fragmentation and maturation and correlation with a) Sperm Kruger and WHO morphology and b) fertilization rate and embryo quality determined by fragmentation and maturity pregnancy outcome and implantation.

The ART outcome was evaluated in 2 groups of patients: Group I patients with < 30% sperm fragmentation and Group 2 with patients with sperm fragmentation > 30%. The analysis statistic was elaborated with ANOVA and Bonferroni’s test.

Results Group 1 (< 30% DNA Fragmentation)/ Group 2 (> 30% DNA Fragmentation) (p)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Age</td>
<td>35.6 ± 4.2</td>
<td>31.7 ± 4.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Male Age</td>
<td>37.3 ± 5.5</td>
<td>43.3 ± 4.7</td>
<td>0.001</td>
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<tr>
<td>Oocytes retrieved</td>
<td>10.22 ± 6.1/11.50 ± 5.4/n.s.</td>
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<tr>
<td>Fertilization Rate (%)</td>
<td>51 ± 2.5/66 ± 3.0/p &lt; 0.001</td>
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<tr>
<td>Semen sperm concentration (MILL/ML)</td>
<td>19.83 ± 22.6/6.2 ± 5.7/p &lt; 0.001</td>
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</tr>
<tr>
<td>Sperm Kruger morphology (KRUGER)</td>
<td>5.7 ± 4/1.9 ± 2/1.1/n.s.</td>
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<tr>
<td>Nuclear Sperm Maturity (%)</td>
<td>76.22 ± 9.9/57.33 ± 20.4/p &lt; 0.001</td>
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<tr>
<td>DNA Sperm Fragmentation (%)</td>
<td>15.11 ± 7.3/47.6 ± 22.03/p &lt; 0.001</td>
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<tr>
<td>Implantation Rate (%)</td>
<td>1.71 ± 0.64/1.68 ± 0.4/n.s.</td>
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<td></td>
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<tr>
<td>Pregnant (%)</td>
<td>8/17 (47%)/4/1 (36.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>2.1 ± 1.04/2.16 ± 1.02/n.s.</td>
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</table>

Discussion The results obtained in this analysis indicate a correlation between the Sperm DNA Fragmentation, Maturity, OMS criteria’s and Kruger morphology. These data also show that sperm with elevated DNA fragmentation can normally (albeit at a lower rate) fertilize, however both the implantation rates and the pregnancy outcome are impaired.

W-095 Sperm biology
Association between seminal oxidative stress, sperm parameters and the sperm deformity index

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Introduction Oxidative stress in semen arises from an imbalance between reactive oxygen species (ROS) generation and the scavenging ability of seminal antioxidants. Oxidative stress influencing male fertility has been well documented. In our study we try to find a correlation between seminal parameters (motility, sperm count and morphology) of 129 infertile men with some of oxidative markers.

Materials and Methods Our work focuses on 129 patients consulting for infertility. They were classified according to World Health Organization (WHO) criteria and divided into four groups: normozoospermia, asthenozoospermia, teratozoospermia and oligozoospermia. These specimens were designated for oxidative stress analyses. Seminal zinc and mineral elements (calcium and Magnesium) concentrations were determined using flame atomic absorption spectrophotometry (FAAS). Selenium levels were estimated by electrothermic atomic absorption spectrophotometry (EAAS). The amount of Malondialdehyde Acid (MDA) in seminal plasma was measured by thiobarbituric acid reaction method.

Results and Discussions Our results reported that mean seminal concentration of magnesium was significantly greater in fertile men compared to infertile patients. But, mean calcium concentration was significantly lowered in fertile controls. Also, MDA concentration showed a significant increase in infertile group. We estimated the probability to have an anomaly in seminal parameters, according seminal concentrations of Zn, Se, Ca, Mg and MDA and we noted a significant probability to have asthenozoospermia for the calcium and MDA. For motility, the probability to have teratozoospermia was only with MDA. Concerning the index of deformity we demonstrated that the extension varied from spermatozoa to another according the subject. We noted an extension of 4% for the abnormal spermatozoa and 5% normal subjects.

Conclusions Our data indicate that ROS could be considered a good predictor of male factor infertility in all infertile men. These results support the notion that the high levels of semen ROS detected in some infertile men are due primarily to excessive generation of ROS and not to defective antioxidant defenses.

IFFS 2010 – Poster Abstracts
An experimental model was created in our lab to study the effect of testicular torsion on biochemical and histological changes that occur following torsion and detorsion in various time intervals. The torsion was applied for a period of 30 minutes. The testicular tissue was reperfused for the duration of 1 hour, 24 h, 48 h and 1 week in different groups of rats and compared with sham operated controls. Histopathological changes observed in testicular biopsy specimens showed sloughing and tissue disorganization during the torsed period (Phase I) followed by marked sloughing, disorganization, death of cellular elements (spermatids and spermatocytes, spermatagonia) (Phase II) following reperfusion in different time intervals leading to the formation of multinucleated giant cells (sertoli cells) (Phase III). The biochemical changes observed in the testis samples of experimental animals were there was an increase in lactate dehydrogenase activity (LDH) as a marker of anaerobic glycolysis. The decline in inorganic phosphate levels and the fall in the content of phospholipids and protein levels indicate a possible membrane damage. This damage appears to be due to Reactive Oxygen Species (ROS) measured as increase in MDA levels, with an initial increase in the activity glucose-6-phosphate dehydrogenase activity and a fall in glutathione (GSH) content the known antioxidant defense mechanism. These changes observed during torsed period and following reperfusion clearly indicates ischemia-reperfusion (IR) injury bring about irreversible changes in testicular function reflected by the histological changes observed as loss of spermatocytes, spermatogonia, spermatids replaced by multinucleated giant cells and the biochemical changes that ensued. The experimental model created in our laboratory is reproducible, viable and reliable. Using this model we are trying to study Gene expression and protein phosphorylation pattern with the period of >48 hrs < 1 wk. The reason for the study is one week recovery following detorsion in rat models denotes the appearance of multinucleated giant cells (sertoli cells engulfing dying cells). On the other hand, after 48 hours of recovery following detorsion dying cells are remarkable and easily noted as a reduction in numbers of spermatogenic complements without the appearance of multinucleated giant cells.

**W-096 Sperm biology**

**An experimental testicular torsion model to envisage testis as an organ of oxygen-antioxidant paradox**

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The sperm motility is important for achieving fertilization of the oocyte. The intracellular calcium ([Ca²⁺]i) is an ubiquitous messenger in the intracellular signalling of human spermatozoa and is considered the most important factor involved in the regulation of sperm motility. In this study we determined the relationship between the [Ca²⁺]i and sperm motility in “non-capacitated” spermatozoa. Sperm from healthy donors were recovered from Percoll gradients. [Ca²⁺]i was measured by spectrofluorometry in sperm suspensions loaded with Fura 2. The [Ca²⁺]i was altered by incubation in different concentrations of extracellular Ca²⁺ (above or below normal) and in presence of inhibitors of plasma membrane Ca²⁺ ATPase (quercetin or eosin), endoplasmic reticular Ca²⁺ ATPase (thapsigargin) or Na/Ca²⁺ exchanger (KB-R76943). Sperm motility was determined in parallel experiments by Computer Assisted Sperm Analysis (CASA). The results show that:

1. The increase of [Ca²⁺]i above 140 nM, induced by an increase in the extracellular Ca²⁺ concentration, reduces the sperm motility.

2. The inhibition of plasma membrane Ca²⁺ ATPase (PMCA), by quercetin or eosin, reduces sperm motility in 36% and 60%, respectively.

3. The inhibition of endoplasmic reticular Ca²⁺ ATPase (SERCA) increases [Ca²⁺]i and reduces the sperm motility (80%).

4. The Na/Ca²⁺ exchanger (NCX) inhibition induces an increase of [Ca²⁺]i and reduces sperm motility (26%).

These results provide evidence that in “non-capacitated” sperm an increase of [Ca²⁺]i above basal level reduces sperm motility. In conclusion, sperm motility is modulated by [Ca²⁺]i, which is regulated by different transport mechanisms such as PMCA, the SERCA and NCX. This information is a novel contribution to the understanding of human sperm physiology and could be useful both to design male contraceptive drugs based in the sperm motility inhibition and drugs to improve men fertility by increasing sperm motility.
W-099 Sperm biology
Protection of human spermatozoa from hydrogen peroxide-induced DNA and membrane damage by exogenous gangliosides

Gayella M.,1 Gargy-Vrhovac V.,1 Lipovac V.,2 Antica M.3
1Vuk Vrhovac University Clinic, Department for Cell Biochemistry, Zagreb; 2Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb; 3Ruder Bokovic Institute, Division of Molecular Biology, Zagreb, Croatia

We have previously demonstrated the protective effect of gangliosides, the sialic acid containing glycosphingolipids, against sperm injury caused by reactive oxygen species (ROS). The aim of this study was to determine whether exogenously added ganglioside GT1b could protect human spermatozoa from DNA fragmentation and increased apoptosis induced by in vitro exposure to hydrogen peroxide (H2O2).

Single-cell gel electrophoresis (Comet assay) was used in the assessment of sperm DNA integrity and the transport of H2O2 across the cell membrane and sperm cell viability after exposure to H2O2. H2O2 was assessed by means of flow cytometry using ROS sensitive fluorescent dichlorodihydrofluorescein diacetate (DCFH-DA) dye and Annexin V in combination with propidium iodide labeling.

The obtained results showed that in vitro supplemented GT1b (100 µM) significantly reduced DNA damage induced by 200 µM H2O2 across the cell membrane and sperm cell viability after exposure to H2O2. H2O2 Flow cytometry revealed that ganglioside GT1b completely inhibited the passage of H2O2 through the sperm membrane.

In conclusion, the results gave evidence on the ability of ganglioside GT1b to protect human spermatozoa from H2O2-induced damage. Reduced DNA fragmentation and apoptotic changes due to decreased H2O2 cell content suggest that ganglioside GT1b, rendering sperm membrane more hydrophobic, acts as an inhibitor of H2O2 diffusion across the sperm membrane.

This study received financial support of the Ministry of Science, Education and Sports of the Republic of Croatia (Grants 045-0000000-0174; 0022-0222148-2125 and 0022-0222148-2125). This study was funded by the Croatian Science Foundation under the project No. 156-7-004-004.

W-100 Sperm biology
Influence of paternal DNA damage on the reproductive outcome

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Numerous exogenous and endogenous factors can damage the sperm, and sperm DNA damage during paternal sperm inheritance or in the male germ cells is responsible for the increased risk of several genetic and non-genetic diseases. The purpose of this paper is to review the literature on the effects of paternal DNA damage on the reproductive outcome.

W-101 Sperm biology
Treatment of cervical insufficiency abortion by autologous human peripheral blood mononuclear cells, modern trend

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1Ali Shams University/Helipolis Research Center, Cairo; 2Institute Kafel El Sheikh University, Department of Veterinary, Kafel El Sheikh, Egypt

Objective To test the efficacy of outpatient intracavitary injection of peripheral blood mononuclear cells in the treatment of habitual abortion cervical insufficiency.

Design Pilot study.

Setting Heliopolis research center and Heliopolis Hospital, Cairo, Egypt.

Intervention Preparation of peripheral blood mononuclear cells, transvaginal ultrasound, IL-8, collagenases in the cervical mucus, Aquaporins in the cervical smear before PBMJ injection at the time of the delivery.

Main Outcome Measures Primary outcome was delivery of full term fetus.

Results Obstetric outcome: full term delivery occurred in 48 cases (96%), abortion 1 case (2%), Preterm delivery 1 case (2%), vaginal delivery in 40 cases (80%), cesarian section in 9 cases (18%). No fetal or maternal complications were reported.

Conclusion Treatment of cervical insufficiency habitual abortion by cervical injection of autologous human peripheral blood mononuclear cell (PBMCs) is safe, effective, and cheap with positive fetal and maternal outcomes, but more cases and randomization is needed before elucidation the effectiveness of the procedure.

W-102 Stem cells
Microarray analysis of gene expression (MAGE) for induced hepatocyte differentiation from either AF-MSC and BM-MSC

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Affymetrix human U133A microarrays were used to analyze eight RNA extracts: 2 amniotic fluid mesenchymal stem cells (AF-MSC), BM-P9 and BM-P11, RA MSC), BM-P9) and BM-P11, and 2 hematopoietic-like cells after induced differentiation from BM-MSC- AHD (BM-P9) and BM-P11), 2 bone marrow mesenchymal stem cells (BM-MSC)- BM-P9) and BM-P11), and 2 hematopoietic-like cells after induced differentiation from BM-MSC- AHD (BM-P9) and BM-P11).

After normalization of DNA microarray results was done, we selected differentially expressed genes using the following criteria: p < 0.05 and fold change > 2. A total of 2,685 genes were shown differentially expressed between BM-MSC and induced hepatocyte-like cells. To explore the potential biological functions of these genes, we uploaded these 20 genes into MetaCore database and analyzed the different functional networks that contained 6 root genes. After normalization of DNA microarray results was done, we selected differentially expressed genes using the following criteria: p < 0.05 and fold change > 2. A total of 2,904 genes were shown differentially expressed between AF-MSC and induced hepatocyte-like cells. Then these genes were ranked by fold changes from the largest (log ratio at –7.60) to the smallest (log ratio at ~7.60). Finally, we would like to compare the change of gene expression between the induced differentiation from AF-MSC to hepatocyte-like cells (AF-AHD) and that from BM-MSC to hepato-
W-103 Pre-implantation embryo Relevance of LIF and EGF on mouse preimplantation embryo development

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Introduction Recent evidence suggests that Leukemia inhibitory factor (LIF), a member of interleukin-6 family has biological actions on preimplantation embryo development. Also it is established that Epidermal growth factor (EGF) is a strong mitosis-promoting agent that improve the preimplantation embryo development by increasing the cell metabolism and proliferation. The purpose of the present study was to investigate the effects of these factors in combination together on preimplantation embryo development.

Materials and Methods 6–8 weeks old NMRI mice were super ovulated by injection of 10IU PMSG and 10IU hCG 46–48h later. The mated mice were killed 46 hours after hCG injection, oviducts were flushed and 2-cell embryos collected and divided randomly to 4 groups as following: Control, treatment 1 (LIF), treatment 2 (EGF), treatment 3 (LIF + EGF). In each group the embryos were cultured in an incubator at 37°C with 5% CO2 and 90% humidity for 72 h. The state of embryo development was evaluated in 24, 36, 48, 60 and 72 hours following culture.

At the end of culture, cell apoptosis was studied by the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) technique.

Results Significant difference was detected in the rate blastocyst formation after 36 hours in the LIF and LIF+EGF groups (p < 0.05). This difference was also seen in the rate of hatching (p < 0.05) and average of total cell number (p < 0.05) after 72 hours. In comparing the apoptotic index, there was no significant difference between the control and treatment groups.

Conclusions The findings in this study suggest a beneficial effect of LIF and EGF on blastocyst formation, hatching and its total cell number in vitro.

W-104 Pre-implantation genetic diagnosis PGD for gender selection: What is the percentage of embryos available and not available?

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Preimplantation genetic diagnosis (PGD) for gender selection, is an authorized treatment in Lebanon, but still debated in many countries. The literature in this field is not developed due to restriction of legislation in many countries. PGD in general have his limitation due to the lost of some embryo and the quality of embryo for biopsy.

In PGD for gender selection, we can obtain in some cases embryo from opposite desire sexes. The aim of our study is to evaluate the percentage of cases where we only obtain embryos from opposite desire sexes.

Materials and Methods In this retrospective study, we performed 83 PGD cycles for gender selection from 2004 to 2009 from 5 different centers ours (1) and others centers (2, 3, 4, 5). 3 centers (1, 3, 4, 5) do the biopsy and send the blastocure and one center (2) send the embryo after ICSI and the biopsy is done in our center (1).

All our patients desire male for gender selection. They all have had at least one or two previous girls from natural conception or ART. Biopsy where done at day 3 with acid Tyrode in our center and Visys probe (actually Abbot) XY and 18 was used for the reading. The result was given on Day 4.

Results 272 Blastomeres have a clear signal with good quality embryos for transfer.

We excluded in this study lost blastomere, non hybridized blastomere, not fixed blastomere and suspicious signal not sure and when we have an incomplete signal (for example X alone).

We observed 138 embryos XX (51%) and 134 embryos XY (49%).

The distribution of all couples was:

- 8 cases with only XX (10%)
- 21 cases with only XY (25%)
- 13 cases with 50% of their embryos XX and 50% XY (16%)
- 41 cases with 10 to 90% of XY (49%) respectively.

Conclusion In 1 of 10 cases we are in the impossibility to replace the embryo of desire gender. This information need to be discussed with the couple before doing the PGD cycle and written in the inform consent form. More studies from other centers are needed to confirm these preliminary results.

W-105 Ovarian stimulation Combination of clomiphene citrate to GnRH antagonists for ovarian hyperstimulation in poor responders: our clinical experience

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Background Various protocols of ovarian stimulation have been proposed for optimizing IVF/ICSI results in women presenting with a poor response to controlled ovarian stimulation; however, achieving a good response to stimulation for this category of patients still remains a challenge.

Materials and Methods A non-randomized prospective trial was performed in patients with 1 or more previous failed IVF cycles in which 3 or less oocytes were retrieved, using > or = 300 IU of gonadotrophins/day. We have used the combination of 100–150mg clomiphene citrate (from Day 2 to 6) to GnRH antagonists (0.25 mg of cetrotrelax daily starting on day 6 to 9) administered as soon as endometrial thickness achieved a limit of 7 mm. Number of follicles aspirated, oocytes obtained, quality of embryos at day 2, cancellation rate and existence of fetal heart as 7 weeks’ scan were the main outcomes.

Results Mean number of follicles were 4 (3–7), oocytes obtained 3 (1–7), mature embryos at Day 2 1 (1–3), 1 cycle was cancelled due to the existence of < 2 follicles for HCG administration and in 1 case fetal heart was positive at 7 weeks scan.

Conclusion The addition of clomiphene citrate to GnRH antagonists in poor responders undergoing IVF treatment is an alternative before these women end to oocyte donation, surrogacy or adoption. The small number of our study population does not permit us to come to firm conclusions.
W-106 Economic issues
Constructing an artificial neural network (ANN) to predict IVF outcomes

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Background Predicting the outcome of in vitro fertilisation (IVF) treatment is an extremely semantic issue in Reproductive Medicine. As discrepancy among reproductive centers’ results still exist and the literature is daily filled with new methods aiming to foresee the desired outcome, there is a clear need for constructing systems to assist/replace human mind.

Materials and Methods Our aim was the construction of a learning machine, that is an artificial neural network (ANN) capable to predict IVF outcomes with high accuracy. Our trial was focused at first in collecting the results obtained from 300 IVF cycles run at the Assisted Reproduction Unit of “Attikon University Hospital” from 2005 to 2009. As inputs data were considered the following parameters: age of the woman, duration of infertility, sperm concentration, number of unsuccessful IVF attempts, basal FSH and AMH levels, daily dosage of gonadotrophins required to reach oocyte recovery, number of follicles before aspiration and retrieved oocytes, number of matured embryos at day 1 and 2, miscarriage rates and severity of ovarian hyperstimulation syndrome (OHSS).

An ANN model construction was attempted based on the popular back propagation of the error algorithm. Initially the data have been pre-processed, i.e. all data inputs were mapped to numbers and uniformly scaled to be into a range between 0.01 and 0.99. Additionally the output of the network will be mapped to 0.99 for positive pregnancy result and 0.01 for negative. In the next step the data set will be separated into 2 groups: the training set, composed from 50% of the positive and 50% of the negative cases randomly selected and the test set, composed from the remaining cases. The third step is the selection of the model architecture: in our case a three layer ANN has been selected. The input layer involves 10 neurons, the output layer 1 neuron and the number of neurons composing the hidden layer will be determined such as the network will have improved generalization capability on the training set. Finally the trained system will be evaluated by its performance on the “unknown” test set.

Results Up till now, training of the ANN is still carried out. The training was planned to carry on using regular clinical data as these have been evaluated in the everyday routine of our IVF Unit. It is expected to have improved predictive power compared to human decisions due to 2 reasons: first, medical data have been mapped to numbers and therefore there will be no subjective evaluation and second, the selected ANN model is highly capable to map non-linear relations between the inputs and the output, thus capturing relations among the data that is not possible to be encapsulated by traditional statistical models, such as tree classifiers and linear regression.

Conclusion We aim to show that the proposed ANN architecture promises good generalization potential and thus it can be a useful tool in the daily routine of the laboratory for the prediction of IVF outcome.

W-107 Ovarian stimulation
Standard addition of low molecular weight heparin during ovarian hyperstimulation in women over 40 undergoing IVF: our preliminary results

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Background Infertile patients over the age of 40 are generally considered to have a low chance of success with assisted cycles despite high numbers of embryos transferred. Chances of pregnancy decrease with increasing age. As women exceed 40 years old, pregnancy and live birth rates fall with concurrent rising miscarriage and cycle cancelation rates.

Materials and Methods 22 women were down-regulated with GnRH-analog starting on cycle day 21 until oestradiol was < 50 pg/ml and no ovarian cysts > 20 mm diameter were present on transvaginal ultrasound. Gonadotrophins were initiated using a standard weight-based stimulation protocol based on FSH and AMH concentrations on early follicular days, patient weight and prior stimulation cycles, if any. When at least 2 follicles reached a mean diameter of 18 mm without oestradiol concentrations measurements, human chorionic gonadotrophin was administered and oocyte recovery was planned 38 hours later. Low molecular weight heparin (3500 IU/daily) s.c. was started from the day of GnRH administration and discontinued only when the pregnancy test was negative; otherwise, it was stopped from each woman’s obstetrician after 7 weeks of pregnancy. Cause for infertility was the advanced age of women, while sperm parameters and ovulation studies were normal. Number of follicles retrieved and matured embryos for transfer as well as cancellation and pregnancy rates consisted the outcome measures.

Results Median numbers were: 4 follicles retrieved, 1.2 matured embryos for transfer at day 2, as well as 4/22 (18.18%) and 2/22 (9%) cancellation and pregnancy rates, respectively.

Conclusion The addition of heparin of low molecular weight to the hyperstimulation regimen does not offer much to the success rates of IVF.

W-108 Embryo
Frozen-thawed single blastocyst transfer achieve high pregnancy rates concurrently low multiple gestations

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Introduction In generally, transferred multiple embryos in IVF program was recognized easiest and most effective methods improved pregnancy rates. However, that was not as improved as expected and also inducing high multiple pregnancy rates. We working to reduce the number of embryo transferred and keep pregnancy rate high in these some years.

Materials and Methods We investigated 6,230 cycles of frozen-thawed single blastocyst transfer between September 2007 and December 2008. The average age of the patients was 38.9 ± 4.5 years. All oocytes retrieved using clomiphene-based minimal stimulation or natural cycle IVF using GnRH agonist for final oocyte maturation. Retrieved oocyte were inseminated conventionally or ICSI. Normally fertilized embryos were cultured individually in a drop of 20 ml of culture medium. All developed blastocyst were once vitrified using Cryotop® methods for subsequent use in frozen-thawed blastocyst cycle. Frozen-thawed blastocyst transfer performed in spontaneous natural or hormonal replacement cycle. All embryo transfer procedures were performed under vaginal ultrasound guidance. Clinical pregnancy was defined after intrauterine gestational sac was confirmed, and the numbers of live birth newborn were counted.

Results Clinical pregnancy and live birth rates per transfers were 42.9% and 31.1% in total, 54.1% and 44.2% (29 years old), 55.1% and 45.5% (30–34 years old), 47.6% and 36.6% (35–39 years old), 32.8% and 18.8% (40–44 years old) and 18.8% and 7.0% (45 years old) respectively. Overall monogynic twinning rate was only 1.12% (30/2,673) under the single blastocyst transfer policy. And singleton live birth rate per transfer was 30.7% as high as live birth rates.

Conclusion Frozen-thawed single blastocyst transfer achieves high pregnancy and live birth rates with low risks of multiple gestations. Our patients population are advanced age including previous failed treatment cycle. For these patients frozen-thawed single blastocyst transfer is effective choice, especially for high aged group > 45 years.
W-109 Male infertility
Inhibin B and anti- Müllerian Hormone as markers of persistent spermatogenesis in men with non-obstructive azoospermia: a meta-analysis of diagnostic accuracy studies

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Introduction
A non-invasive test that could predict the presence of sperm during a testicular sperm extraction (TESE) procedure in men with non-obstructive azoospermia would be of profound clinical importance. Inhibin B (Inh-B) and anti-Müllerian hormone (AMH) have been proposed as direct markers of Sertoli cell function and indirect markers of spermatogenesis.

Methods
A search was conducted in the electronic databases MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials from inception through June 2009. 35 different studies reported data on the predictive value of one or more index markers (serum Inh-B: 32 studies, seminal Inh-B: 5 studies, serum AMH: 2 studies, seminal AMH: 4 studies) were included in the systematic review. 9 studies, which had serum Inh-B as index marker, met the predefined criteria and included in the meta-analysis.

Results
Serum Inh-B demonstrated a sensitivity of 0.65 (95%-CI: 0.56–0.74) and a specificity of 0.83 (CI: 0.64–0.93) for the prediction of the presence of sperm in TESE. When the pre-test probability of 41% was incorporated in a Fagan’s nomogram, resulted in a positive post-test probability of 73% and a negative post-test probability of 23% for the presence of sperm in TESE.

Conclusions
Inh-B cannot serve as a stand-alone marker of persistent spermatogenesis in men with NOA.

W-110 Reproductive endocrinology
Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing in vitro fertilization: a meta-analysis

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Introduction
The aim of this study was to investigate whether thyroid autoimmunity (TAI) is associated with increased risk for spontaneous miscarriage in subfertile, euthyroid women undergoing in vitro fertilization (IVF).

Materials and Methods
A literature search was conducted in the electronic databases MEDLINE and EMBASE from inception through to June 2009 in order to identify eligible studies. 4 prospective studies, which reported data on 1,098 subfertile women undergoing IVF (141 with TAI and 957 controls) were identified and included into the meta-analysis.

The main outcome measure was the miscarriage risk ratio (RR). Clinical pregnancy rate and delivery rate were examined as secondary outcome measures.

Results
Euthyroid, subfertile women with TAI undergoing IVF demonstrated significantly higher risk for miscarriage compared to controls (4 studies – fixed effects RR: 1.99; 95%-CI: 1.42–2.79; p < 0.001). No significant difference in clinical pregnancy and delivery rates was detected between groups.

Conclusions
Based on the currently available evidence, it appears that the presence of TAI is associated with an increased risk for spontaneous miscarriage in subfertile women achieving a pregnancy through an IVF procedure.

W-111 ART clinical
The sperm motility index (SMI) after swim-up can be used to predict the occurrence of normal fertilization following conventional insemination during assisted reproductive technology

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Purpose
The technology to analyze the concentration, motility and velocity of sperm during swim-up is available by computer to aid in assisted reproductive technology (ART) treatment. By using this technology, the evaluation of sperm has become easy and stable. Recent reports have mentioned that the Sperm motility Index (SMI), which was the original index defined by the company that developed this technology, has had a positive effect on the rate of fertilization. SMI combines sperm concentration, motility and velocity, but no cut-off value has been developed to predict normal fertilization for conventional insemination during ART treatment. In the present study, we attempted to set SMI values to predict the occurrence of normal fertilization during conventional insemination.

Materials and Methods
Between February and November of 2009, a number of patients (n = 101) who received an oocyte pick-up in our clinic were enrolled in the present study. Patients who received intracytoplasmic sperm injection (ICSI) treatment due to male infertility were excluded. We evaluated the relationship between SMI and the incidence of low fertilization before and after sperm swim-up following conventional insemination. Low fertilization was defined as a rate that fell below 40%.

Results
The average age of the patients was 35.5 years, and the mean fertilization rate for conventional insemination was 62.0% (338/545). The incidence of low fertilization during the study period was 23.8% (24/101), and the SMI before swim-up was not related to the incidence of low fertilization. However, among the patients recording a SMI of less than 300 after swim-up, the incidence of low fertilization was 42.8%, and this rate was significantly higher than that among patients who recorded a SMI of more than 300 (18.8%; p = 0.209). Moreover, the patients who recorded a SMI of less than 400 after swim-up experienced a significantly higher incidence of low fertilization (37.1%) than those who recorded a SMI of more than 400 (37.1%, p = 0.214).

Conclusion
The 40% of patients who recorded a SMI of less than 300 after swim-up experienced low fertilization. Therefore, these patients should routinely be referred to ICSI treatment if they are to avoid an occurrence of low fertilization.
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