Key Lectures

K-1
New treatment in PCOS

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Conventional treatment of PCOS was focused on hyperandrogenism. But decreasing insulin resistance seems to work better.

- Vit D deficiency decrease insulin resistance and Vit D supplement can help in improvement of PCOS signs and symptoms.
- Chromium piconilate decrease insulin resistant and improve menstrual cyclicity in PCOS patients
- N acetyl cystein (NAC) could be either to improve induction ovulation/or to help for biochemical changes like lipid profile, HOMA IR
- Orlistat, as an antilipid medication can improve lipid and sugar profile and decrease BMI and gives better menstrual cyclicity
- Myoinositol and Dchiro inositol also decrease insulin resistance and has good metabolic effects on lipids, insulin and FBS, BMI, BP
- Metoformin still play important role in alleviating signs and symptoms of PCOS and we can use it either for priming before ovulation induction or for decreasing insulin resistance and change of metabolice statements of the patients.

At the end we can conclude that PCOS is a multiorgan disease and has a viscious cycle, and with breaking this viscious cycle in any area especially in insulin resistance, we can have success in treatment.

K-2
Endocrine disrupting chemicals and male infertility

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Endocrine disrupting chemicals (EDCs) influence our health, including male reproduction. There is an increase in male reproductive disorders: the most striking one is the increase in testicular cancer in most western countries. The number of young men with testicular cancer has doubled in the last 20 years and continues to increase. At Erasmus MC in Rotterdam we focus our research on the potential causes of this increase in testicular abnormalities and found the early stages of this malignancy are already present in very young boys, operated for non-descended testes. This strongly indicates that the origin of this disease is related to testicular development during early. The most logical explanation for this increase is environmental influences on testicular development during the first 3 months of pregnancy. The development of male genitalia is mainly influenced by genes and by testosterone produced by the fetal testis. Disruption of this development will result in "Testicular dysgenesis syndrome" (TDS). This maldevelopment of the testis and male genitalia result in 5 birth defects and health problems later in life: 1) male infertility, 2) small testes with low testosterone production, 3) testicular maldescent, 4), hypospadias and 5) testicular cancer. We see a rapid increase in all of these abnormalities, which can only be explained by environmental influences. Many chemicals in our environment have a structure similar to estrogen and androgens and may block the effects of fetal androgens on the androgen receptor. The can pass the placenta easily and influence male development during early pregnancy. Common examples of EDCs are Bisphenol A (BPA), dioxins, furans, PCBs and different pesticides. The number of disease potentially linked to EDCs is substantial and include;

- Obesity and diabetes;
- Female reproduction (premature ovarian failure, female infertility);
- Male reproduction (cryptorchidism, hypospadias, male infertility, male hypogonadism);
- Hormone-sensitive cancers in females (breast cancer in young woman);
- Hormone sensitive cancers in men (testicular cancer);
- Thyroid diseases;
- Neurodevelopment and neuroendocrine systems disorders.
- Immune system defects (asthma, food allergies).

Many of these diseases will only appear later in life, thus making it difficult to prove a causal relation with prenatal EDCs exposure. However, in the last years both animal and human studies have strongly indicated this relationship. Animal studies and observation in wildlife provide strong evidence that manmade chemicals can disrupt the hormone dependent pathways responsible genital development. A decline in sperm quality has been reported in many industrialized countries. Studies in humans now also show a negative effect of EDCs on male fertility. In a recent studies the effects of pesticides exposure resulted in a decline of sperm quality of 30% later in life compared to men that were not exposed.

K-3
Paternal antigen specific Treg cells play important roles for successful implantation and maintenance of pregnancy

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Fetus is a semi allograft to maternal host, therefore tolerance system is necessary for successful implantation and maintenance of pregnancy. Regulatory T cells (Treg), especially paternal antigens (PA) specific Treg, play a central role for induction of tolerance. To

detect the PA specific Treg, female BALB/C mice were mated with male DBA/2 mice. Mls 1a antigen on DBA/2 mice is recognized by the T cell receptor Vβ6, so CD4+Foxp3+Vβ6+cells are recognized as PA-specific Treg cells. Interestingly, ki67+PA-specific Treg cells were significantly increased in uterine draining lymph nodes before implantation, and increased in uterus after implantation. Seminal plasma priming is necessary for induction of PA-specific Treg in uterine draining lymph nodes by the differentiation of dendritic cells (DC) into tolerogenic DCs. In human, Helios-positive thymic derived functional regulatory T cells are decreased in decidua of miscarriage cases with normal fetal chromosomal content but not in miscarriage cases with abnormal fetal chromosomal content, suggesting that decreased functional Treg cells at fetomaternal interface might induce miscarriage in human. Fetus is a complete allograft to maternal host in oocyte donation (OD) pregnancy, and it has been reported that OD pregnancy is a risk for preeclampsia. We have shown that accumulation of decidual Treg cells, T cells, NK cells and monocyte were impaired and vascular remodeling was also impaired in OD pregnancy, therefore not only Treg cells but also T cells, NK cell, and monocytes also play important roles for the placentation in human.

K-4
Human endogenous retrovirus, Syncytin 1 (HERV-W), in human pre-implantation embryos, embryonic stem cells and following differentiation to trophoblast in vitro

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Endogenous retroviral elements (HERVs) play a role in normal cell function as well as disease. Syncytin 1 is an HERV envelope protein crucial for cell fusion to form syncytiotrophoblast, essential for embryo implantation and placental development and is highly expressed in human placenta. We investigated the expression of Syncytin 1 in human pre-implantation blastocysts, and human embryonic stem cells (hESCs) before and after spontaneous differentiation. To induce trophoblast, hESCs were incubated in conditioned medium supplemented with BMP4 or FGF4 growth factor. Synctin 1 was expressed on trophectoderm cells of pre-implantation blastocyst and on trophoblast outgrowth of embryos cultured in vitro. Additionally, there was expression of Syncytin 1 in the inner cell mass. Undifferentiated hESCs exhibited very low or absent expression of Syncytin 1. During culture hESCs differentiated to trophoblast and exhibited cell-cell fusion and syncytium formation. Syncytin 1 was expressed on trophoblast as determined by mRNA, immunofluorescent localisation and western blot analysis. This study reveals Syncytin 1 protein expression in the pre-implantation human blastocyst which increases during differentiation to trophoblast, suggesting a role for this HERV at the very earliest stages of human embryo development.

K-5
Advanced cellular technologies in reproductive biology

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Stem cells are considered as potentially new therapeutic agents for the treatment of infertility. Stem cells could be stimulated in vitro to develop various numbers of specialized cells, including male and female gametes. So, they have a potential application in reproductive medicine. During the past 10 years, considerable advances in the derivation of male germ cells from pluripotent stem cells have been made. In addition, stem cell-based strategies for ovarian regeneration and oocyte production have been developed in laboratories for the future clinical therapies to overcome infertility in women. Apart from these conditions, mitochondrial diseases are clinically heterogeneous groups of diseases that arise as a result of dysfunction of the mitochondrial respiratory chain. While some of these disorders only affect a single organ (i.e., the eye in Leber optic neuropathy), many involve multiple organs and present with prominent neurologic and myopathic symptoms. Recently, scientists removed the nucleus from a healthy donor egg and replaced it with a nucleus taken from the egg cell of a woman who carries a rare neurological disease called Leigh syndrome, leaving the donor's healthy mitochondria intact. The scientists then fertilized the modified egg with the father's sperm before implanting it into the mother's uterus. The resulting baby was born in April 2016. Therefore, strategies to create three-parent babies offer mothers a way to have a child without metabolic disorders caused by defective mitochondria.

K-6
The transition from genetic to genomic medicine

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Genomic Medicine today is built on a history of clinical, scientific and technological contributions. Over the 60 years since the discovery of the structure of DNA and the introduction of chromosome analysis for diagnostic purposes an increasing range of services has been available to benefit patients with genetic disorders and

their families. During the last 20 years the vast explosion in knowledge accompanying the development of genetic technologies has allowed medical genetics to have a much greater impact on medicine from a vastly increased range of diagnostic tests, even therapies for some conditions as in the case of Marfan syndrome, the product of the pioneering work of Hal Dietz at Johns Hopkins Hospital. This coincides with the birth of the term Genetic Medicine which underlines the central role that genetics plays in medicine today. More recently the more general term 'precision medicine' hints to a specific treatment which is given on the basis of a germ line or somatic mutation (for example in a tumour) or a drug prescribed in doses based on a genotype i.e. pharmacogenetics. The new technologies enabling targeted capture and massively parallel sequencing of individual genomes/exomes, known as Next Generation Sequencing (NGS), have resulted in major discoveries initially on small clinically well characterized patients. On the other hand new developmental pathways have been elucidated through Genome Wide Association Studies (GWAS, like in the SardiNIA population project) and some disorders with overlapping clinical features shown to be due to mutations in functionally related genes (modifiers), may become amenable to treatment by similar molecules, as it might occur in the case of Spinal Muscular Atrophy. From 2010 onward the emphasis has shifted from discovery to diagnostic applications. Families of individuals with unknown disorders are being offered exome sequencing of trios (mother, father, child) or targeted testing using large panels of appropriate genes being offered to patients with specific disorders such as retinal dystrophy, cataract, epilepsy etc. Interestingly results of diagnostic applications of NGS indicate that there is a much wider phenotypic spectrum associated with mutations in many genes than was suspected from initial clinical definition and Sanger sequencing and many centers are now introducing whole exome sequencing (WGS) into diagnostic practice. Medical Genetics as a clinical specialty is constantly changing and the last 20 years have seen a massive increase in referrals of conditions generally regarded as common complex disorders such as breast and bowel cancer and some cardiac diseases. The first challenge is to separate out those families with a 'monogenic subset' of the disease which are the group which our current services can best help. Meanwhile large scale research efforts such as in the Icelandic and Sardinian populations study have been making progress looking for genetic variations- generally of small effect- which contribute to the pathogenesis of common disorders and the new technologies are rapidly contributing to this research too. Finally the great change in the practice of Medical Genetics is the introduction of non-invasive prenatal testing (NIPT) for a greater range of chromosomal and single gene disorder, a field pioneered by Diana Bianchi. Alongside with the development of genetic technology during the last 60 years, some educational initiatives were developed in America and Europe, like the Short Course in Medical Genetics started by V.A. McKusick in Bar Harbor, Maine-USA, in 1960, the European School of Medical Genetics, which later became European School of Genetic Medicine started in Sestri Levante, Italy, in 1988 (now located in Bertinoro) and the Latin American School of Human and Medical Genetics started by Roberto Giugliani in Caxias do Sul, Brazil, in 2005. During the same years these courses and schools trained thousands of young geneticists coming from all over the world and contributed to the transition from Medical Genetic to Genetic Medicine and eventually to Genomic Medicine.

K-7
When ART is indicated in endometriosis

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Endometriosis is a disease known to be detrimental to fertility. A significant number of women with endometriosis will eventually seek ART, namely in vitro fertilization (IVF) with or without intra-cytoplasmic sperm injection (ICSI) for conception. Between 17 and 44% of women with endometriosis will have endometrioma. The exact pathophysiology of endometrioma related to infertility is still unknown. It can be detrimental to fertility directly by distorting tubo-ovarian anatomy or indirectly by invoking inflammatory and oxidative on the oocytes resulting in poorer quality oocytes. Surgical treatment of endometriosis and endometrioma prior to IVF/ICSI is widely practiced even though very little evidence exists to provide robust guidance to clinicians. More recent studies have generated some concern that the surgical treatment on endometrioma could be detrimental to ovarian reserve and subsequently adversely affect IVF/ICSI reproductive outcomes. The possible adverse outcomes associated with the presence of endometrioma during IVF/ ICSI have also not been studied. The risks of surgery and its potential damage to ovarian reserve have to be balanced with the complications associated with the persistence of the endometrioma during IVF/ICSI. While an earlier meta-analysis reported worsened in vitro fertilization (IVF) outcomes for women with mild endometriosis compared with other infertile women, subsequent larger meta-analyses have consistently reported that women with minimal to mild endometriosis have similar live birth rates after IVF compared with women without endometriosis. In contrast to mild endometriosis, Stage III/IV disease appears to negatively impacts ART outcomes. Three systematic reviews reported lower oocyte retrieval,

implantation, and pregnancy rates for women with advanced endometriosis undergoing IVF compared with women with mild endometriosis. However, women with advanced disease, particularly those with endometriomas, often have lower ovarian reserve and produce fewer oocytes for recovery, which reduces ART success. The impact of diminished ovarian reserve becomes more pronounced in women of older age whose declining egg quality is associated with greater embryo aneuploidy. In women with poor ovarian reserve, such as those with advanced age and severe endometriosis, we discuss the option to pursue ART with donor oocytes. There is no evidence that ART increases the recurrence of endometriosis. In addition, the use of ART in women with endometriosis does not appear to increase the risk of poor birth outcome, particularly preterm birth.

K-8
Epigenetic-associated regulatory mechanisms are involved in maternal communication with gametes and embryo

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Maternal communication with gametes and the embryo(s) has been extensively studied over the last two decades, in human, and other species that utilise internal fertilisation as a means of reproduction. Several reports from my laboratory and others, have comprehensively described the interaction of both gametes and embryos with the female reproductive tract, from the time of gamete deposition through to embryonic implantation. It is becoming evident that the environment within the female reproductive tract can influence these interactions and as a result, can affect the epigenetic profile of offspring. Despite all advances taking place in this field, it is still not known how communication between mothers, gametes and the embryo is mediated. One potential explanation is the existence of receptors in the female reproductive tract for recognition of gametes and embryos, similar to those pathogen recognition receptors known to exist in the innate immune system. Toll Like receptors (TLRs) are a good example of such receptors. While the existence of such receptor molecules seems to be a logical idea, to date, there is no concrete evidence for their presence in the female reproductive tract. As the interaction of gametes and embryos with the maternal tract is known to be accompanied by changes in the transcription profiles of maternal tract epithelia, another potential mechanism of regulation of these interactions might be via epigenetic and epigenetic-associated regulatory mechanisms. Investigations performed in my laboratory suggest that extracellular vesicles and microRNAs are involved in the regulation of maternal communication. A better understanding of the mechanisms involved in regulation of maternal tract interactions with gametes and the embryo will help us to devise novel diagnostic tools and therapeutic approaches to treat infertility. In addition, these investigations will support our knowledge of how epigenetic regulatory molecules affect intercellular communications in all body systems.

K-9
Conservative management of women's reproductive organ's pathologies to preserve their future fertility

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Abstract not received.

K-10
ART in PCOS patient

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Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. The main characteristics of PCOS are anovulation, hyper androgenism and polycystic ovary morphology many therapeutic strategies have been used to Restore ovulation:
First line treatment: 1) Life style modifications (diet and exercise) in obese patient, 2) Induction ovulation: clomiphene citrate.
Second line treatment: LOD and exogenous gonadotropins after the fail two fist treatments the third line treatment sagest to patient is ART. In women with PCOS, supra physiologic dose of gonadotropins use for COH provoke the development of a large cohort of follicles of uneven quality, which leads to poor fertilization and lower cleavage, pregnancy and live birth rate protocol for ART: long protocol: OCP + GnRH agonist and gonadotropin (FSH)
Antagonist protocol: FSH + antagonist and use GnRH agonist for LH surge trigger
And in vitro maturation (Ium) adjuvant therapy be for ART: OCP, Metformin, LOD and IVF is controversial

K-11
Laparoscopic myomectomy, for unusual myoma locations

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Uterine leiomyomata (UL), also known as fibroids, are benign tumors of the uterus and the leading cause of hysterectomy in the United States, accounting for $1.2 billion in hospital expenditures annually. Most women develop myomas during their lifetimes; however, 80% of them are asymptomatic. When symptoms are determined to be caused by myomas, a number of management options exist that include "watchful waiting", medical therapy, surgery, or more recently uterine artery embolization and focused ultrasound. Uterine myoma is a common gynecologic disorder occurring in 20-50% of women of late reproductive age and preservation of fertility is the primary concern. The first lesson physicians must learn is that if the patient is asymptomatic, no treatment is necessary. The presence of an abdominal mass is not an indication for hysterectomy or myomectomy unless it is of significant concern to the patient. Symptoms vary in severity and include pelvic pain, abnormal menstrual bleeding, and pregnancy complications. The etiology of UL is poorly understood. Increasing incidence of diagnosed UL during reproductive years and decreased incidence with menopause suggest the role of sex steroid hormones. Recently, laparoscopic myomectomy has been advocated because of its small operative wound, short hospital stay, quick recovery, and outcome comparable to traditional laparotomy. Myomectomy, either abdominal or laparoscopic, is an approach particularly suited for those women who wish future fertility. It seems clear that, in well trained and experienced hands, well-selected patients can have myomectomy performed under laparoscopic direction. Very large myomas are not as suitable for the laparoscopic approach, but laparoscopic myomectomy up to 20 cm has been reported in literature, which solely depends on surgeons ability. There are no universally accepted criteria regarding number and size of myoma to be removed laparoscopically but as our techniques, especially suturing techniques and instruments for laparoscopy advance, our ability to do more complicated cases of laparoscopic myomectomy increase as well. Before laparoscopic myomectomy uterine mapping is mandatory, because the surgeon does not have sense of palpation during procedure, in order to have successful laparoscopic myomectomy the surgeon should answer the following questions before surgery.

- How many myomas are there?
- Where is the exact location of myomas?
- How is the distance of myoma from cavity?
- Is uterine cavity distorted?
- Are we able to perform operation?

Laparoscopic myomectomy is a challenging procedure and the most challenging part of this procedure is suturing. The goal of suturing is to restore myometrial integrity, prevent hematoma formation, prevention of defect and dehesence in myometrium and adhesion prevention. If any one of these goals is not met during procedure the future pregnancy would be in danger. Skill of surgeon is the most important factor for successful operation. In video clip the laparoscopic myomectomy in unusual myoma locations will be displayed.

K-12
Treatment option in PCO resistant to clomiphene citrate

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Clomiphene citrate (C.C) is the traditional first-line treatment of chronic anovulation that characterize polycystic ovary syndrome (PCOS). However, 20-25% of patients fail to ovulate even with maximal dose of C.C. The next step in these patients is to use insulin sensitizer like Metformin which improves ovulation and pregnancy outcome. Adjuvant therapy plus C.C such as gonadotropin induce ovulation with adverse effect of ovarian hyperstimulation syndrome (OHSS) in some patients. Other adjuvants such as Bromocryptin, glucocorticoid or HCG may also improve the outcome. Moreover, operative strategies like drilling or cauterization of ovary may beneficial and help to increase the ovulation in some group of patients.

K-13
A fil rouge links numerical to structural chromosome abnormalities via chromothripsis

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Non-disjunction at maternal meiosis is the primary cause of spontaneous abortions as documented by extensive epidemiological studies showing trisomies in more than 50% of sporadic miscarriages. However in a number of trisomic products of conception trisomy rescue may occur restoring the normal number of chromosomes, eventually leading to a more favorable condition for survival. We hypothesize that most constitutional supernumerary marker chromosomes (sSMC) are the relic of the supernumerary chromosome, resulting from a chromothripsis event leading to partial trisomy rescue. According to this model, chromothripsis is initiated by anaphase lagging of the supernumerary chromosome followed by its massive fragmentation within a micronucleus. The loss of some fragments and the gluing together of others might be the final outcome of the original supernumerary chromosome. To investigate the correctness of this hypothesis, we are sequencing a number of sSMCs by paired end 30x whole genome and analysing the haplotype of the sSMCs and the chromosomes from which they originate in the trios. The results in the first seven cases show that most sSMCs are formed by non-contiguous regions of

the original chromosome or by contiguous ones with portions repositioned in inverted order after NHEJ, as it is expected for chromothripsis events. Moreover, though the parental origin of the sSMC resulted to be either maternal or paternal, the chromosomal portions outside the sSMC itself resulted to be biparental in the case of sSMCs of maternal origin or in hetero/isodisomy for sSMCs of paternal origin. These data demonstrate a link between numerical and structural anomalies and that the devastating effect of trisomies may not limited to prenatal life.

K-14
Testicular tissue cryopreservation: Factors affecting the outcome and cumulative pregnancy rate in cases of non-obstructive azoospermia

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Abstract not received.

K-15
Ultrastructural markers of quality in human metaphase ii oocytes cryopreserved with media containing different macromolecular supplements

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Cryoprotective agents (CPAs) are essential components in freezing solutions, but may also disrupt the meiotic spindle and organelles. Together with conventional CPAs, protein supplement is known to preserve cell structure. Cortical granule (CG) exocytosis requires a healthy plasma membrane and cytoskeleton. This process may be affected by cryopreservation as a result of shrinkage during CPA addition leading to subjacent localization of CG and resulting in release of their contents because of plasma membrane fusion after rewarming. The present study has been carried out in order to verify whether type and concentration of protein supplement included in freezing solutions affect the ultrastructure of human metaphase II (MII) oocytes cryopreserved by slow freezing and therefore optimize cryopreservation conditions. Forty supernumerary MII oocytes were donated by consenting patients (aged 28-36) enrolled in an IVF program. Thirty-four oocytes were cryopreserved using slow freezing with 0.2M sucrose, 1.5 M 1-2 propanediol and either serum or Plasma Protein Solution (PPS) in the freezing mixture. Six oocytes were used as fresh controls. Oocytes were cryopreserved with 20% (n=12) and 10% (n=10) serum or 10% PPS (n=12). Samples were fixed by 2 hr after thawing and prepared for light and transmission electron microscopy (LM and TEM) for ultrastructural analysis. By LM, both control and cryopreserved oocytes appeared rounded and with uniform distribution of organelles. By TEM, mitochondria-smooth endoplasmic reticulum aggregates and small mitochondria-vesicle (MV) complexes were the most numerous structures found in all oocytes. Only in a few cryopreserved oocytes, irrespective of macromolecular supplement, numerous large MV complexes were found, probably due prolonged culture (3-4 hr) before cryopreservation. Amount and density of CG appeared abnormally reduced in all samples. Different degrees of vacuolization were present in the ooplasm of cryopreserved, but not fresh oocytes. Extensive vacuolization was present only in a minority of oocytes cryopreserved with serum (16.6% of the oocytes supplemented with 20% serum and 20% of the oocytes supplemented with 10% serum), whereas a higher number (66.6%) of oocytes supplemented with 10% PPS were largely vacuolized. In conclusion, this study confirms: 1) slow freezing generally maintains the oocyte structure; 2) premature CG exocytosis and vacuolization are both markers of cryodamage; 3) prolonged culture before cryopreservation may cause enlargement of MV complexes. This study also originally reveals that serum supplementation induces good preservation of the ooplasm avoiding extensive vacuolization. This approach can be considered of interest for different cryopreservation methods adopted in assisted reproductive treatments. In fact, the matter of protein supplement in different formulations and concentrations is still debated both for culture media supplementation and vitrification solutions.

K-16
From POR to low prognosis concept: A new proposed stratification by the POSEIDON working group

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The incidence of poor ovarian response (POR) during ART has generally been reported to vary from 9-24%. Until the establishment of the ESHRE Bologna criteria for POR (2011) no strict criteria to define POR existed, hampering the conclusions drawn from clinical trials and meta-analyses. However, after their introduction even the Bologna criteria were criticized of describing a heterogenous group of patients with different success rates after ART. Importantly, no clinical recommendations for handling of the POR patient were given. In contrast, The Poseidon Group recently proposed a new stratification system in an attempt to further define the group of low prognosis patients, taking into account ovarian reserve and age, which are

the two most prominent key factors to predict success in ART (Alviggi et al., 2016; Humaidan et al., 2016). In this stratification system four different sub-groups of low prognosis (POR) patients are defined as well as the suggested matching protocols and regimens, which might increase the success rate of the patient. Moreover, Poseidon introduces a new measure for successful ART treatment, namely, the number of oocytes needed in each specific patient to obtain one euploid embryo for transfer. The so-called Poseidon Calculator which is currently being developed will enable clinicians to calculate this new measure, also taking into account site specific parameters. During this lecture an updated review of strategies and adjuvants as well as future therapeutical options for the low prognosis (POR) patient will be presented. Although, the handling of the poor responder patient still represents a therapeutic challenge, there might be some light "at the end of the tunnel".

K-17

Array-comparative genomic hybridization (array-CGH): The first report of its clinical application for preimplantation genetic screening (PGS) in Iran

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Embryonic aneuploidy contributes to reduced implantation rates, IVF cycles failure and early pregnancy loss. The main treatment strategy for patients with these complications is to improve the endometrial receptivity and the quality of the embryos transferred. For this respect further IVF attempts coupled with preimplantation genetic screening (PGS) is an acceptable alternative treatment. The goal of PGS is to identify and select the most competent (euploid) embryos for transfer. The use of PGS with array-CGH technology, which assesses the whole chromosome complement, at day 3 embryo biopsy markedly improves live-birth rate, increases implantation rates and reduces aneuploid pregnancy and miscarriage rate. This is the first report of application of array CGH - based aneuploidy screening on embryos at day 3 stage in Iran. During the period from 2015 until this report, a total of 139 patients undergoing their ICSI/PGS cycles at Assisted Conception Unit, Laleh Hospital, Tehran, Iran. The indications for PGS included repeated implantation failure (RIF) (≥3 IVF failures), Recurrent Abortion (RA) (≥3 pregnancy losses), advanced maternal age (AMA), history of chromosomally abnormal pregnancy, child and family history of genetic disorders, Abortion (<3 pregnancy losses), IVF failure (<3 IVF cycle failures). The indications for undergoing PGS in 28.8% of patients were RIF, 30.9% recurrent abortion, 5.8% AMA, 5% history of genetic disorders, 12.2% Abortion, 10.8% IVF failure and 6.5% IVF failure together with Recurrent Abortion. According to a-CGH investigation 175 (19.68%) out of 889 embryos biopsied yielded at least one euploid embryo and 714 (80.32%) were aneuploid. Therefore 54 cycles after PGS were cancelled. Of the 85 cycles in which euploid embryos were transferred after PGS, 24 cycles were positive for Beta-hCG (28.23%). The PGS effect was evaluated for each indication separately. The pregnancy rate in RIF, RA, AMA genetic history cases and abortion were 11.53% (3/26), 37.5 (9/24), 40% (2/5), 20% (1/5) and 55% (5/9), respectively. In total, among 24 pregnancies in PGD cycles, 75% resulted in live births, 16.66% continued pregnancy, 8.33% abortion, 4% IUFD and 4% pregnancy termination. Some treatment strategies offered for couples with RIF, RA and AMA are to improve the quality of the embryos transferred and the receptivity of the endometrium. Treatment recommendations should be evidence based, and if the prognosis of further IVF attempts is considered poor, alternative treatment options (such as oocyte and embryo donation or surrogacy) may be necessary. Based on these preliminary data, application of array CGH -based PGS can provide a more accurate chance of success for women of abortion, advanced maternal age and recurrent pregnancy loss because it is related to favorable clinical outcomes. Furthermore, in women with RIF, after appropriate investigations to rule out other underlying cause for the repeated failure and if, the actual source of the problems lies with the embryo, PGS using microarray-CGH is valuable and it should be offered.

K-18

A co-culture system supplemented by hormones and growth factors as model to reduce granulosa cell apoptosis in vitro

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In mammalian ovaries, more than 99% of follicles during follicular development undergo atresia, by which they are removed from the pool of growing follicles. The formation of an atretic follicle seems to be initiated by granulosa cell (GC) apoptosis, through complex molecular mechanisms involving tumor suppressors, apoptotic proteins and survival factors, whose relative expression levels in GCs determine whether an ovarian follicle will grow or undergo atresia in the late preantral stage. Several strategies have been tried to increase GC survival in order to improve oocyte maturation and fertilization potential, as the co-culture systems. However, yields are still suboptimal. Therefore, in this study we aimed to evaluate the effect of hormone and growth factor supplementation to reduce GC apoptosis by using an in vitro co-culture system made of pig GC multilayers adhering to the basal lamina and associated with cumulus-oocyte complex (COCGs). In vitro culture (IVC) was done in standard condition, with FSH and EGF supplementation. COCG morphology, apoptotic rate, caspase expression levels and surface ultrastructure were determined at the end of IVC. Respect to sampling, an increased granulosal apoptosis was found in control and EGF-supplemented groups, associated to caspase activation. In contrast, the percentage of apoptotic cells was significantly reduced by FSH supplementation, as also demonstrated by the expression of inactive procaspases. The pro-survival effect of FSH was strengthened by EGF, as evidenced by a significant reduction of GC apoptosis and high levels of Procaspases. Multilayers of round-to-ovoid cells were connected between each other and to the basal lamina by cytoplasmic projections. Reduction of the microvillar coverage, rarefaction of cytoplasmic projection, presence of cytoplasmic blebbing and degenerating/atresic GCs were observed in control and EGF-supplemented groups. Differently, FSH induced the formation of an abundant mucinous matrix. Blebs and atresic areas of GCs were rarely observed. In the group supplemented by FSH and EGF, GCs were richly covered by microvilli and connected by numerous long cytoplasmic projections. Degenerative phenomena were rarely observed. In conclusion, supplementation of EGF and FSH can significantly reduce GC apoptosis in a co-culture system made of pig GC multilayers with the COC anchored on them.

K-19
Evaluation of fertility preservation with GnRH agonist in breast cancer cases treated with cyclophosphamide as an chemotherapy drug

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25% of breast cancer cases are detected during premenopausal period and the number of young women suffering from breast cancer is increasing in the world, especially in Iran. Preservation of fertility and ovarian function leads to improved quality of life of these patients. The aim of this study was to evaluate the effect of gonadotropin releasing hormone (GnRH) agonist on menstrual reverse in breast cancer cases treated with cyclophosphamide regimen. This randomized clinical trial (RCT) was conducted on 42 adenocarcinoma cases. 21 case group with GNRH analog and 21 patients of control ones without GNRH. Mean age of patients was 37±5 yr (range 25-45 yr). Patients with primary stages to stage II (T2N1M0) whose histology reports were ER/PR negative were enrolled in this study. All the enrolled patients were candidates for cyclophosphamide (600 mg/m^2), adriamycin (60 mg/m^2), and taxoter (75 mg/m^2) chemotherapy regimens. Spontaneous menstrual reverse occurred in 90.5% of patients receiving diphereline at 3-6 months after treatment which occurred in 33.3% of control cases. In control group, 14.3% (3 cases) had oligomenorrhea and hypomenorrhea during chemotherapy and 19% (4 cases) had spontaneous menstrual reverse at three to six months. It should be noted that there was a significant difference between controls and cases ($p<0.001$). This difference was insignificant in cases younger than 35 yr ($p<0.594$). In 100% of patients older than 35 yr who received diphereline, spontaneous menstrual reverse occurred during six months after chemotherapy, but this occurred in only 20% of controls ($p<0.001$). Mean serum level of follicle stimulating hormone (FSH) and luteinizing hormone (LH) during and at three months after therapy was significantly lower in cases in comparison with controls, but serum level of estradiol was significantly more in cases three months after chemotherapy ($p<0.001$). GnRH agonists significantly improve ovarian function and fertility. They also lead to spontaneous menstrual reverse in negative ER/PR breast cancer cases.

K-20
Endometriosis in adolescence

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Endometriosis is a chronic progressive disease that is common in women and it's incidence is higher between in infertile women. The exact mechanism of pathogenesis of endoemtriosis is unknown but recently there is some evidence that showed the origin of endoemtriosis was begun before adolescence and even 5% is seen in newborn. Recent work indicates that NUB represents a significant biomarker for events that can occur later-on during adolescence. Indeed, clinical

studies have shown that "neonatal menstruation" constitutes a sign of fetal distress during late pregnancy, reflecting a stage of endometrium development that may subsequently have an impact on the reproductive life of the adolescent and the young adult. Endometriosis is suggested in adolescents with a history of chronic pelvic pain or dysmenorrhoea resistant to medical treatment. All phenotypes of early/superficial and advanced forms of endometriosis are found in adolescents, including ovarian endometriomas and deep endometriotic lesions. Recent evidences suggest that adolescent endometriosis can be a progressive condition, at least in a significant proportion of cases. There isn't any curative treatmen and long term recurrence is still a significant problem. The most frequently reported treatment approach is a combination of surgery and postoperative hormonal treatment with the different suitable hormonal therapy with the aim of ovulation suppression. There is controversy in treatment to whether surgical treatment should be considered at an early stage before more severe lesions develop or surgery should be avoided as much as possible to prevent multiple operations in the long term.

K-21
Influence of ovarian endometrioma on fertility

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Endometriosis is a benign chronic gynecological disease, defined as the presence of endometrial tissue outside the uterine cavity. Prevalence has been estimated to reach 10-15% of reproductive-aged women, and 25-50% of infertile women. Assisted reproductive technologies (ART) are commonly offered for managing endometriosis-related infertility. ART results, however, vary according to reports, with some showing identical outcome as in endometriosis-free counterparts, and others describing lower pregnancy rates. In this context of discordant ART results in endometriosis, there is no consensus about the possible impact of the endometriosis phenotype on ART outcome. The use of the laparoscopic approach, in particular the "stripping" technique, has been questioned because it could involve excessive removal of ovarian tissue with loss of follicles. In spite of the laparoscopic removal of ovarian endometriotic cysts is a tissue-sparing procedure. Laparoscopic approach is still the less invasive technique in treating ovarian endometriosis; it appears aggressive in terms of injuring the residual ovarian tissue. Cumulative spontaneous pregnancy rate (cSPRs) is significantly lower in women treated by expectant versus surgical management. In addition, the presence of OMAs, both in patients treated with expectant or surgical management, caused a further decrease of cSPRs. Endometriosis is associated with lower oocyte yield, lower implantation rates, and lower pregnancy rates after IVF. However, the association of endometriosis and IVF outcomes is confounded by other infertility diagnoses. Endometriosis, when associated with other alterations in the reproductive tract, has the lowest chance of live birth. In contrast, for the minority of women who have endometriosis in isolation, the live birth rate is similar or slightly higher compared with other infertility diagnoses.

K-22
Modern diagnostic and management techniques of endometrioma and DIE

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The most important presentation of endometriosis are DIE and endometrioma. These patients could have either infertility, pain symptoms, or may be asymptomatic. Modern management of these common lesions will be discussed in the presentation. Literature was reviewed from year 2000 up to now and those studies with a good level of evidence were selected. Also our publications and researches were reviewed and according to these data a guideline for diagnosis and management of endometrioma and DIE in patients with infertility, pain symptoms, or asymptomatic patients will be presented and discussed in my presentation. According to literature review and our extensive experiences with these patients, those with infertility and endometrioma should be selected very carefully for either ovum pick up or surgery and for women with DIE and infertility alone surgery is debated. For patients with endometrioma and pain, they should be operated but there are many factors before surgery which should be considered. For patients with DIE and pain, the definite treatment is surgery. For women who are asymptomatic and had DIE or endometrioma, surgery should be only considered only for those who had major damage to vital organs such as ureter, kidney and bowel. Patients with endometrioma or DIE who presented with infertility or pain should be individualized according to their hormonal profiles, ovarian reserve tests, and imaging technique and then treatment should be considered for them. I will discuss our guideline for management of these patients in details in my presentation.

K-23
Endometrial receptivity in endometriosis

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The reasons for repeated In-vitro Fertilization (IVF) failure may be defective endometrial receptivity,

embryonic chromosomal abnormality, or multiple factorials. Defective endometrial receptivity may be due to molecular dysregulation or morphological disruption. The pathogenesis of endometriosis-related infertility is still unclear. Numerous mechanisms proposed for fertility impairment in these patients, including altered folliculogenesis, ovulatory dysfunction, poor oocyte quality, luteal phase defects, reduced fertilization, and abnormal embryogenesis as well as reduced receptivity due to compromised endometrium. Many molecular and immune characteristics of eutopic endometrium from women with minimal/mild disease and even women with moderate/severe disease appear to differ from that of disease-free women. Global gene expression, histone modification patterns *HOXA11* expression in eutopic mid-secretory endometrium is very different in patients with endometriosis, which may contribute to endometriosis-associated infertility. Herein we discuss about some new proposed reasons for reduced endomtetrial receptivity in endometriosis.

K-24
Adenomyosis and infertility

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It is uncertain whether adenomyosis is a cause for infertility in women, however women with adenomyosis has a 28% reduction in the likelihood of clinical pregnancy at IVF/ICSI compared with women without adenomyosis. MRI has enabled a noninvasive diagnosis and showed that medical, surgical, or combined treatment can restore fertility in women with adenomyosis, an indirect proof of an association. Concerning the relationship between adenomyosis and infertility, many theories have been proposed as follow;

- Intra-uterine abnormalities
- Disturbed Uterine peristalsis
- Destruction of normal myometrial architecture and function
- Altered intra-endometrial steroid metabolism
- Abnormal inflammatory response
- Altered expression of estrogen and Progestrone receptors
- Altered uterine oxidative stress environment
- Impaired implantation (Lack of expression of adhesion molecules, reduced expression of implantation markers, and altered function of genes for embryonic development.)

All these abnormalities in the endometrial environment seem to contribute to subfertility. Several attempts have been made to restore fertility in adenomyosis patients, the oldest being gonadotropin-releasing hormone agonists coupled to conservative surgery. Also, uterine artery embolization and MRI-assisted high-intensity focused ultrasound ablation have been tried with some degree of success.

K-25
Ovarian resserve and endometriosis: cryopreservation and the role of surgery

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Endometrium is one of the most frequent pathologies in gynecologic surgery Despite extensive research of endometriosis there are important controversies in this area. Although Laparoscopic cyst excision is considered the best treatment in terms of lower recurrence but the diminished Ovarian reserve after surgery is an important factor that effects on decision making of the patients. There is3 reason in favor of surgery. Lower follicular density in affected ovary due to focal inflammation and fibrosis that is related to the sie of endometrioma. 2) gonadotoxic effect of endometrioma on the surrounding follicles. Another persuasive argument favoring surgical excision of endometrioma relates to the dangers of expectant management such as ovarian torsion, cyst rupture, progression of endometriosis, or the threat of ovarian malignancy. The reason against the ovarian surgery consists of: 10 presence of ovarian parenchyma in 40% of cases of endometrial cystectomy. Surgical excision of endometriomas leads to damage of healthy cortex and a decline in AMH that appears progressive. The important factors for the amount of ovarian damage after cyctectomy is age >35, size and method of cyst wall removal by stripping. There is much debate over the treatment of these cysts in infertile women, particularly before use of ART. Nevertheless, evidence exists that supports the presence of an endometrioma does not appear to adversely affect IVF outcomes, and surgical excision of endometriomas does not appear to improve IVF outcomes. The advantage of oocytes collection for fertility preservation prior to surgery relates to detrimental effect of surgery on ovarian reserve. Although removing healthy ovarian tissue away from endometriomas can deteriorate ovarian reserve but collection of the cortical tissue that are attached loosely to the capsule and ovary is a good chance for attached cryopreservation during endometrioma surgery is a good chance for FP. Freezing embryos or unfertilized oocytes seems to be the most convenient technique of fertility preservation for women suffering from endometriosis. It does not affect ovarian reserve and offers a real chance of future pregnancy when a good amount of oocytes or embryos has been stored.

K-26
An introduction to Stem Cell Biology Research Center in Yazd

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Stem Cell Biology Research Center has started its activities as a stem cell laboratory in Yazd Clinical and Research Center for Infertility since 2006 with a work on human gonocytes which was presented in ISSCR2007. Later studies on stemness of TESE-derived cells and foreskin derived cells were started and presented in ISSCR2008. Results of TESE study was published in 2016 in MRD journal. In parallel other works on dental pulp stem cells, application of rat bone marrow derived mesenchymal stem cells in treatment of stroke in animal model were published as original articles. Derivation and characterization of Yazd human embryonic stem cells (YAZD1-3) were other projects which were done in the center. Later facilities of the labs were transferred to a bigger building with two cell culture lab, one molecular biology lab, one chemical lab, one imaging room and freezing room with office space to establish the Stem Cell Biology Research Center in the Yazd Reproductive Sciences Institute. At the moment 6 PhD projects and 7 master projects are running in the Center including working with YAZD human embryonic stem cells and tissue engineering. Hereby, we announce that the cells and cell lines which have been produced in the Center are available for other groups according to the Yazd Reproductive Sciences policies. Behrouz Aflatoonian dedicates his share of the studies which he is contributed to Bibi Fatemeh Karbassi.

K-27
Stem cell translational medicine: A bridgable gap between basic science and clinical application

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Stem cell therapy has introduced promising hopes for the treatment of various diseases. On the other hand, clinical utilization of stem cells needs to translate basic sciences and protocols before starting clinical phases by bridging stem cell research into clinical trials. Therefore stem cells translational medicine will open a new horizon in this area of research and practice. Accordingly, there are several risk factors relevant to safety issues of stem cell preparation and transplantation that must be considered in translational phase. For instance; transplantation site reactions, immune responses, biodistribution, ectopic grafting, unintended differentiation into another cell type, tumorigenicity, and lack of functional characteristics. In summary, to conduct clinical stem cell transplantation trials, the safety concerns must be carefully weighed against the potential benefits and all preclinical and clinical researches must be designed to elucidate potential safety concerns before translasting from the bench to the bedside.

K-28
Basic principles of GMP-compliant stem cell manufacturing

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Cell based therapies provide exciting new opportunities to treat incurable diseases. In most cases large number of pure cell population is needed, therefore the isolated cells need to be expanded in vitro before transplantation. In vitro manipulation of cell products requires complex laboratory procedures that increase the risk of possibly adverse events for the recipient. To minimize the associate risks of cell transplantation, adhering to current international standards for clinical grade cell manufacturing is critical. According to the current international regulations and regulations of Iran Food and Drug Organization, cell therapy products should be manufactured under principles of GMP. The main focus of this lecture will be on principals of Good Manufacturing Practice (GMP) which defines optimal quality and safety for cell based products. Among different elements, proper selection of cell processing reagent and appropriate working environment are the most challenging aspects of GMP. Therefore, I discuss about how to select appropriate ancillary materials for clinical grade cell manufacturing. However, different aspects of clean room facility with paying particular attention to facility design, qualification and maintenance will be discussed.

K-29
Biotechnology of human embryonic stem cells from first derivation to robust defined culture for therapeutic applications

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Clinical trials and therapies developed using products (e.g. cells, secretions) originally from pluripotent stem cells (hESCs, iPS) are required to use Good Manufacturing Procedures (GMP) to gain regulatory approval. A history file of each cell line documents all the processes undertaken, and provides a validated report of all the cell line attributes. Practical cell culture methods need to be truly robust and for many applications must ultimately be open to scale-up and automation to meet economic/commercial considerations. Ensuring that batches of cells are of precisely the same quality and function is a critical requirement. Since pluripotent stem cells adapt both functionally and genetically to their local environment in vitro, developing effective methods of manufacture and monitoring is a major challenge. In my presentation I will give an overview of our experience of deriving and maintaining clinical grade hESC lines, focusing on the practical issues faced in the past and those we face in the future.

K-30
Spermatogonial stem cells from chicken and their differentiation potentials

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Spermatogonial stem cells (SSCs) have received considerable attention in science and clinical communities in recent years. Despite high level of biotechnological significance, not many studies have so far been conducted into the derivation, enrichment and characterization of chicken SSCs in vitro. We aimed to investigate the molecular signature and differentiation potential of derived chicken SSCs and also to expand and purify their population in vitro. Non-enzymatic mechanical digestion of testes and culturing of its fragments was used for derivation of SSCs from the testicular tissues. The chicken SSC-like cells were successfully derived and further investigated by differentiation into adipocytes, osteoblasts and neuron-like cells and into spermatozoa. A simple method was established for expansion and purification of chicken SSC populations in vitro. They were subjected to differentiation assays and shown that colony-forming cells maintained their stemness potential in cell cultures with the potential to differentiate into various cell types. This study confirms the efficiency of the used method to achieve optimal culture conditions for chicken SSC derivation. We report here novel insights into the molecular signature of spermatogonia, especially SSCs, in newborn chicken testis and its cell cultures.

K-31
Application of stem cells from different sources for the treatment of reproductive system diseases

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Stem cells have long been proposed for the treatment of congenital and acquired reproductive system disorders including ovary and testis problems. Stem cells from different adult and embryonic origins including bone marrow-derived mesenchymal stem cells (BM-MSC), Umbilical cord matrix-derived stem cells (UCM; WJ-MSC) and Adipose tissue-derived stem cells (ASC). HUCB are randomly harvested from fetus umbilical cord blood and are preserved for further use in liquid nitrogen. WJ-MSCs are simply propagated by enzymatic and explant methods with nearly identical properties which can be used in regenerative medicine procedures. ADSCs can easily be harvested from adult adipose tissue following different surgeries including cosmetic, general and special interventions. Following collagenase digestion, ADSCs can be proliferated in random culture media including serum supplement. Little parallel studies have been carried out to compare mesenchymal and stemness properties of these cells. But a growing body of knowledge is emerging that show there are some differences regarding surface markers and differentiating capacity of these stem cells. Immunogenic property of stem cells which are planned to be used in regenerative medicine is a hallmark which requires close attention. BM-MSCs and ASCs can be harvested from the patients and be used for the treatment of some known diseases in the human and animal models. While WJ-MSCs do not express HLA antigens and are probably immune competent when used as a heterogenic graft. Properties of different MSCs and their probable use in human regeneration especially in reproductive system will be discussed.

K-32
Regenerative medicine in the reproductive system

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Regenerative Medicine makes a great hope for regeneration, repair and replacement of diseased, lost or malfunctioned organs and tissues. It will offer clinicians wonderful abilities for treatment. The triad of cells, scaffolds and stimulant agents are famed in tissue engineering. Stem cells with their pluripotency and potential ability for tissue regeneration, are the main

sources of multifunctional cells for repair and tissue engineering. Scientific approaches to scaffold synthesis and various natural and man-made materials which could carry and support cells, have become an attractive field of research and collaboration among scientists all around the world. Finally, stimulators could conduct the cell fate and tissue growth and development over the basement for the best functioning tissue. This picture has enormous complexity and variability yet; but coordinated medical, scientific and engineering teams could make a bright future, and these progresses need a multidisciplinary approach. Considering Good Manufacturing Product (GMP) rules for stem cell, scaffold and stimulant production, there is a long path to clinical application of synthetic tissues and organs after laboratory tests. According to its prophecy, Yazd Reproductive sciences Institute is trying to have a GMP-approved stem cell line production, various methods of cell therapy research and tissue engineering with a special look to the regeneration of male and female reproductive systems and treatment of special challenging diseases. We hope to be one of the pioneers in reproductive tissue engineering as good as being the pioneer of in vitro fertilization (IVF) in Iran.

K-33
A role of indolamine 2,3-dioxygenase-1 in the chorionic vascular endothelium

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Indoleamine 2,3-dioxygenase-1 (IDO1) catalyzes the first step of the kynurenine pathway of L-tryptophan (L-Trp) degradation. The enzyme has antimicrobial and immunoregulatory activities. Oxidative degradation of L-Trp leads to a local depletion of L-Trp and the formation of Trp metabolites, which both display immunosuppressive effects, including the generation of regulatory T cells and reducing the immunogenicity of dendritic cells. IDO1 has been implicated in the regulation of feto-maternal tolerance. In another aspect, vascular endothelial IDO1 is linked to the regulation of the vasotonus. In the placenta, we find IDO1 localized in the decidual glandular epithelium and in the vascular endothelium of the villous chorion and also in the endothelium of spiral arteries of the decidua. We asked the question whether IDO1 plays a role in the regulation of the tonus of placental vessels, this way contributing to placental perfusion and consequently to placental growth. In this context we also asked whether pregnancy complications such as fetal growth restriction (FGR) and preeclampsia (PE) are pathogenetically linked to a deficiency in placental vascular tryptophan catabolism. L-Trp induced vasorelaxation of ex vivo-perfused placental cotyledons and stimulated pre-constricted placental arteries. This effect was partially blocked by using an IDO1 competitive inhibitor. Vasorelaxation of pre-constricted arteries from the chorionic plate following upregulation of IDO1 by IFNgamma and TNFalpha was found in myography upon exposure to Trp. A decrease in IDO1 protein expression was found by Western blotting in FGR and PE in comparison with pre-term controls. We conclude that L-Trp metabolism by IDO1 contributes to the regulation of the placental vascular tone. Expression of IDO1 is down-regulated at the protein level in FGR and PE placentae, suggesting a possible causal relationship between deficient vascular endothelial IDO1 and pregnancy complications.

K-34
Controlled ovarian hyperstimulation affects the endometrial distribution of the immune cells and reduces the success of ART

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Blastocyst implantation is one of the most important steps in assisted reproduction techniques (ART).The efficiency of this step is determined by three main parameters: endometrial receptivity, embryo quality and a well-balanced embryo-endometrium interaction. It is well known that a successful implantation and achieving suitable results of pregnancy rate depend on a proper embryo and a receptive endometrium interaction. The implantation window is a critical period in which the endometrium has acquired the most proper morphological, cell composition and functional state for the blastocyst attachment under the precise control and regulation of sex hormones. It has been proposed that controlled ovarian hyperstimulation adversely disturbs endometrial receptivity during ART cycles. This phenomena is mediated by the up-regulated concentrations of estradiol and progesterone, leading to morphological, molecular and cellular alternations in endometrium. The cells of the immune system like T cells, NK cells, macrophages, dendritic cells, NKT cells etc., also show cyclic changes in their frequency and localization in endometrium under the guide of sex hormone during the menstrual cycle. The important role of these cells in establishment of a proper interaction between fetus and endometrium, endometrial receptivity and implantation is reported by many investigators. Any alternation in frequency and homing of these undoubted important cells can affect the rate of implantation and ART. In this review the changes in recruitment of the immune cells to endometrium following hormonal alternation during ovarian hyperstimulation will be discussed. We conclude that the changes in endometrial distribution of T cells, NK cells, NKT cells, macrophages and dendritic cells as a result of ovarian

hyperstimulation could lead to reduced endometrial receptivity and success of ART. Considering the advances in embryo cryopreservation techniques and quality of the frozen embryos, we suggest to postpone the embryo transfer procedure for normalization of cell content and receptivity of endometrium.

K-35
New aspects of thin endometrium in ART

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Successful embryo implantation needs a good quality embryo, coincident with a receptive endometrium. Sub optimal endometrial growth is an important step in endometrial receptivity and embryo implantation. Thin endometrium less than 7 mm is correlated to a lower chance of pregnancy. Intrauterine adhesions due to infection or curettage, treatment by oral contraceptives or clomiphene citrate, congenital anomalies, or past history of radiotherapy can lead to thin endometrium. However, thin endometrium is reported in 2.4% of assisted reproductive technology cycles. A thin endometrium sometimes is reported in in vitro fertilization (IVF) cycles in spite of the absence of demonstrable causes. Several strategies to treat thin endomethriun have been studied including extended estrogen, ovarian hyper-stimulation with gonadotropins, low dose aspirin, low-dose hCG, tamoxifen, pentoxifylline and vitamin E, l-arginine, sildenafil, acupuncture and neuromuscular electric stimulation, granulocyte colony-stimulating factor (G-CSF), stem cell therapy and autologous platelet-rich plasma. In spite of the many modality of treatment, most of the options lead to only minor change in the endometrium thickness and subsequent pregnancy, and when this modality fails, patients are eventually candidate to surrogacy.

K-36
The role of varicocele treatment in the management of non-obstructive azoospermic patients

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The varicocele prevalence in the general population is estimated to be 15%; however, the prevalence is 35% among men with primary infertility and 81% among men with secondary infertility. Varicoceles that are detected via physical examination are referred to as clinical varicoceles, whereas those that are >3 mm in diameter and observed only via Doppler ultrasound with the Valsalva maneuver are considered sub-clinical varicoceles. Azoospermia or severe oligospermia occurs in 4-13% of men with clinical varicoceles. The only treatment option for men with non-obstructive azoospermia (NOA) who desire to be biological parents is testicular sperm extraction (TESE) with intracytoplasmic sperm injection (ICSI). The cumulative data reveal that varicocelectomy can improve spermoatogenesis in NOA patients. Although varicocele repair improves spermatogenesis in 39.1% of patients, TESE is inevitable due to inadequate numbers of sperm in some patients' ejaculates and to azoospermia relapse following the recovery of spermatogenesis in other patients. Varicocelectomy increases the micro-TESE sperm-retrieval rate in men who remain azoospermic following varicocele repair. The testicular histopathology may predict the success of varicocele repair. There is a strong association between genetic defects and varicocele-related infertility in men, so it is necessary to investigate the effect of coexisting genetic anomalies on varicocele repair. In light of the currently available data, varicocele repair should be considered before TESE/ICSI in all azoospermic men who have clinically palpable varicoceles.

K-37
The human oocyte ultrastructure in ART. Recent acquisitions

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Assisted reproduction technology (ART) success depends on the oocyte functional and morphological quality. The healthy completion of oocyte maturation associated to the quasi absence of degenerative alterations in the ooplasm is essential for making the female gamete competent for fertilization. ART procedure itself may also alter the morphodynamics of oocyte organelles during maturation and thus may impair oocyte quality. Electron microscopy associated with clinical, epidemiological, biological, molecular and biochemical data, greatly supports the correct evaluation of the oocyte integrity during ART procedures as in vitro fertilization (IVF), cryopreservation (CP) and in vitro maturation (IVM). Evaluation of oocyte quality, especially after CP, is based mainly on the morphological appearance of the oocyte. Phase contrast microscopy (PCM) is currently used for scoring oocyte and embryo quality. However, a good survival as evaluated by PCM may be not related to a good capacity of the oocyte in yielding a competent embryo. The use of electron microscopy to evaluate fine morphological damage is a tool to understand oocyte quality preservation to a higher sensible level. Transmission electron microscopy (TEM), especially when associated with a morph metric analysis, allows an accurate experimental evaluation of fine details of cell microanatomy that can be compromised during ART procedures. Here we will review our most recent ultrastructural studies of the human oocyte subjected to

ART procedures. Oocytes for these studies were obtained after informed consent from women (whose infertility was due to male or disovulatory factors) in fertility age, subjected to ART cycles. The studies were approved by the ethical committee of the institutions involved and followed the current ethical European guidelines for Clinic and Research studies. Controlled ovarian hyperstimulation was induced with protocols using GnRH agonist and rFSH. Only oocytes devoid of any dysmorphism at PCM examination were used for ultrastructural studies. All the samples, used for electron microscopy observations, were fixed in as described elsewhere. The main ultrastructural features of ART oocytes may be summarized as follows. Oocyte ooplasm usually shows uniform distribution of organelles in all protocols. Mitochondrial morphology appears similar between the different conditions. Cortical granules are stratified in a single, mostly continuous row just beneath the ooplasm in well preserved cells, but may alter their distribution and paralleled by zona pellucida hardening in CP oocytes. Microvilli may present a variable degree of modifications. Vacuoles, when present, are frequently associated with lysosomes and correlated with poor quality preservation. Mitochondria-smooth endoplasmic reticulum aggregates and mitochondria-vesicles are sensible organelles complexes and may present heterogeneous morphology and distribution. The MII spindle is quite susceptible to ART procedures. These data showed how ART derived maturing oocytes undergo a complex and coordinated reorganization of its genome, ooplasm and surrounding extracellular matrix that, as seen by electron microscopy, appears to be characterized by neogenesis, modification and redistribution of organelles, membranes and glycoproteins in the ZP. Our ultrastructural studies demonstrated that different fine cellular aberrations may occur in the human oocyte as the consequence of the application of ART protocols (CP and IVM, in particular) and could be co-responsible for ART failures, even affecting early embryo development. The definition and standardization of fine structural markers of quality is mandatory for evaluating ART effects of human oocyte integrity. The goal is giving a contribution to the realization of poorly aggressive protocols that may improve ART viability.

K-38
The immunomodulation by menstrual blood stem cells at the beginning of the road

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There is growing body of evidence demonstrating that menstrual blood stands as a viable source of stem cells. Menstrual blood-derived stem cells (MenSCs) express markers associated with mesenchymal and embryonic origin and have recently been the focus of research for their multi-lineage differentiation potential. They are highly proliferative with stable genetic signature over several rounds of replications and sampling could be repeated periodically in a non-invasive and simplified manner. Despite steadily growing interest for their utility in treatment of several preclinical disease models including but not limited to autoimmune and degenerative diseases, data on their potential interaction with immune system is surprisingly scarce. In a part of our recent investigations, we showed that MenSCs express immunomodulatory molecules previously reported to be secreted by mesenchymal stem cells. We found out that under steady state conditions, MenSCs expressed FOXP3 and upon stimulation with IFN-γ they could express significant amounts of IDO1 and COX-2. They are also able to modulate proliferative capacity of allogenic T and NK cells and effectively hinder optimal generation and maturation of monocyte-derived dendritic cells. In continuation of our studies, we recently showed that there are inherent and immunologic functional differences between MenSCs derived from endometriotic and non-endometriotic women and provided robust evidence on potential involvement of MensSCs in reproductive-related disorders. These results propose that MenSCs are rather immunomodulatory stem cells. To exploit MenSCs full therapeutic potential, more insight is needed to unravel the mechanisms through which these cells affect different arms of the immune system.

K-39
Immunotherapy for women with recurrent spontaneous abortion "Trying to use Human Amniotic Epithelial Cells, Sperm and use of vitamin D3 as a supplements"

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There are various treatments for women whit recurrent spontaneous abortion (RSA) such as *Lymphocyte* immunotherapy, IVIG, *corticosteroids and* etc. However different studies from many clinical trials show a large controversial topics. One may conclude that there is no effective treatment that accepted by majority of clinicians. Immunology department in Isfahan University of medical sciences carried several studies on women with recurrent spontaneous abortion. Our results stated that there is an immunological homeostasis disruption in these women. Also we demonstrate that *Lymphocyte* immunotherapy with paternal *Lymphocytes* is more effective than other therapies especially in women with idiopathic RSA. Considering controversial nature of current treatments, replacement therapy would be necessary. This may be use of other cells e.g. sperm and Human Amniotic Epithelial Cells (HAECs). We focus on these cells

because of their special characteristics. Studies have shown that these cells can induce regulatory T cells and reduction of effector T cells responses. We are studying various aspect of these cells such as the expression of hormonal and non-hormonal receptors, their changes in interaction with leukocytes and vitamin D3 and type of antibody which may produce by contact of these cells with peripheral blood leukocytes.

K-40
Effect of the ovarian reserve in embryonic chromosomal abnormality of the women with recurrent pregnancy loss

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Anti-Müllerian hormone (AMH) is an important clinical marker for ovarian reserve and it is measured as first assessment for couples with infertility or recurrent pregnancy loss (RPL). Previous study reported that more than half of miscarriages are associated with chromosomal abnormalities of the embryo, which causes by lower quality of the oocyte. Women with RPL more likely have diminished ovarian reserve, which could causes poor oocyte quality. It could causes higher embryonic chromosomal abnormalities rate in these women. This should be considering in the evaluation of couples with RPL. Cytogenetic analysis of aborted fetus of women with RPL showed significantly more chromosomally abnormality than in women with one abortion. Greater abnormal embryonic development with cytogenetic defects also was assessed by hystero-embryoscopy. Decreased ovarian reserve is greater in women with RPL than the dependability of their age.