Key Lectures

K-1
Past present and future of human reproduction

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Since the last forty years, Assisted Reproduction Technologies (ART) open a new area for infertile couple. Most of the etiologies of sterility such as tubal blockage, ovulation perturbation or endometriosis can be treated. Ovarian stimulation, in vitro fertilization, intra cytoplasmic injection and freezing approach is now available for both female and male factors. But two black boxes have to be explored: the uterus capacity to implant and the capacity of each gametes and embryos to develop. Genetic, epigenetic and immunological approaches will be the next steps of knowledge in order to increase the results and to give a personalized proposition for each couple.

K-2
The improvement of IVF cycles outcome: A new approach

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Integration of basic science and clinical study of infertile patients result in improving the success rate of IVF cycles. Unfamiliarity and or lack correlation between basic and clinical scientists involved in reproductive sciences lead to ignore some reproductive system disturbances that can affect ART cycles outcome. According to financial and emotional burden of the failure of ART cycles, it seems that the time has come to modify ART from relatively the same form of drug administration for all patients or exam and error on them to design the specific ART cycles based on the initial characteristics of the patient's reproductive system. In this approach, IVF/ET protocols being designed only after close monitoring of each patient's natural cycle to identify; the initial characteristics and disturbances, antral follicular count, follicular and luteal phase length, the patient's endocrine profile, the largest size of dominant follicle, partial or complete rupture of dominant follicle, grading of the endometer, occurrence of premature luteinized or delayed maturity of endometrium, the characteristics of previous induction cycles and the probability of initial or final oocyte atresia. All of these factors need to evaluate and record on predictor sheets to discuss with the immunologist, embryologist and genetic specialist to design a protocol for induction and embryo freezing. Based on the results, this integrated approach can significantly improve the IVF cycle outcome thus it should be offered to the patients to achieve the best possible results.

K-3
Surgical or medical treatment for unruptured interstitial (cornual) ectopic pregnancy? That is the question

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Cornual pregnancy is a rare and most dangerous form of ectopic pregnancy (EP) which is usually treated by corneal excision or hysterectomy. The consequence of corneal location of gestation is usually massive intraperitoneal haemorrhage, necessitating a blood transfusion. Controversies exist between the group of gynaecologists who excise the corneum via laparoscopy or laparotomy and the group of gynaecologists who leave the corneum intact and use drugs (i.e. Methotrexate) for treatment of corneal EP. Expectant management of this type of EP is suitable only for women with low and diminishing levels of βHCG.
Each group claim their way of managing unruptured EP is preferable over the other method. There are advantages and disadvantages in each mode of treatment. Patients with corneal EP usually have signs and symptoms of ectopic gestation later in the first trimester of pregnancy. This is because the location of the gestation allows more room for the growing EP. Therefore the size and the level of βHCG are higher than other types of EP. In fact there are anecdotal reports of term interstitial EP. In view of this fact, these patients with high levels of βHCG are not suitable for medical therapy. Currently laparoscopic surgery is the preferred treatment for EP. There are 2 laparoscopic techniques:

1. Laparoscopic wedge resection of the corneum which involves removal of the myometrium surrounding the interstitial section of the tube. This results in higher risks of uterine rupture in the subsequent pregnancies.
2. A simple, swift and safe (SSS) laparoscopic technique for the treatment of interstitial pregnancy is applying 2-3 vicryl endoloops below the affected corneum incorporating the proximal third of the tube, mesosalpinx and portion of the myometrium adjacent to the corneal EP. This should be done after cornuostomy and suction evacuation of the products of conception in the corneum. This technique is easy to perform by any gynecologist whom has some experience in laparoscopy.

This technique was developed by the author in New Zealand in 1995 on an unexpected corneal EP undergoing emergency laparoscopy. This case was presented at the 26th Annual Meeting of the American Association of Gynaecologic Laparoscopists in Seattle (September 23-28, 1997) and published in the Journal of the American Association of Gynaecologic Laparoscopist (May 1999, Vol. 6, No. 2) as a new laparoscopic approach for the treatment of interstitial ectopic pregnancy. Following this laparoscopic treatment the patient had 3 more pregnancies which were all intrauterine and in the last pregnancy the baby was born by caesarean and tubal ligation was performed as per patient request and consent in 2001. In the author's opinion the above laparoscopic technique is preferred to the medical treatment because it ends the EP and its risks and patients anxiety in one session. Methotrexate may be a reasonable option in selected women with a low βHCG level but is not successful in every interstitial pregnancy.

K-4
Ultrarapid freezing "Vitrification" is the right tool for cancellation of fresh embryo transfer

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Single embryo transfer is becoming increasingly popular in IVF/ ICSI. More IVF/ ICSI cycles therefore include freezing of high quality embryos, and the cumulative effect of such cycles becomes more important. To improve the results obtained using frozen-thawed embryos, the predictive value of embryo and patient characteristics such as ovarian reserve, hormone levels and age play an important role in both cases whether the women treated with Oestradiol/ progesterone or undergo natural cycle transfer. Although, embryo quality indicators revealed sometime morphologically and numerically inferior embryo cohorts after cryopreservation, the clinical pregnancy rate is higher in cycles using thawed embryos compared with fresh embryos. Moreover, subsequent logistic regression analysis controlled for differences in embryo quality and revealed significantly greater probability of clinical pregnancy with thawed embryos when compared with fresh embryos, suggesting a negative effect of ovarian stimulation on endometrial receptivity. The aim of this study is to discuss an idea of cancellation of a fresh embryo transfer and put on an alternative method which is the frozen thawed embryo.

K-5
Sociocultural influences on fertility in the Middle East: the role of parental consanguinity, obesity and vitamin D deficiency

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Infertility is worldwide acknowledged as a major health concern. Although infertility prevalence appears to remain unchanged since the 1990s, significant regional differences have been reported in infertility prevalence. The prevalence of infertility in women of reproductive age has been estimated to be one in every seven couples in the western world and one in every four couples in developing countries. Geographical, sociocultural/ religious and ethnical dissimilarities contribute to these global variations of infertility prevalence. Infertility has a major impact on family stability in many cultures, especially in developing countries, where childlessness can impact sociocultural status. Moreover, it is important to realize that most fertility treatments are based on studies performed in Western countries. The purpose of this review is to critically appraise the existing evidence regarding the association between female fertility and relevant sociocultural factors in Middle East countries focusing on aspects such as parental consanguinity, obesity and vitamin D deficiency. There may be reason to believe that in addition to the current standard evaluation of infertile couples, region-specific counselling and treatment modalities are required.

K-6
Ultrasonography screening in obstetrics in perinatal medicine. What, when and by who?

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Ultrasonography represents the most significant advance in obstetric diagnosis and clinical management in the past 40 years. Ultrasonography in pregnancy is a simple, painless and harmless examination used in everyday practice for the present diagnosis. The largest risk of antenatal sonography is probably misdiagnosis. A false positive diagnosis of a malformation may lead to parental anxiety and these errors can be corrected by a second examination in a tertiary referral center. A missed diagnosis (false negative) remains undetected unless the patients undergoes for a second examination for another indication. These limitations are often gestational age dependent. But if a significant congenital anomaly is recognized at delivery one of the patients question is: «Could we have seen this on ultrasound before delivery?» Obstetrics sonography should be performed at an appropriate gestational age by an experienced practitioner.
The ACOG and the AIUM have published guidelines for the basic ultrasound examination in pregnancy.

This basic examination is performed most often for the purpose of biometry and the establishment of gestational age. Various descriptive terms have been used to identify such a detailed study including level II comprehensive, extended and targeted. This targeted study is performed for the detection of fetal anomalies in women at risk for having a malformed fetus. The pregnant patient expects to have information about baby's health and in case a congenital anomaly is present she wants to kwon the prognosis, the treatment and the recovery. Routine use of ultrasound in low pregnancies has been offered for the decrease of labor inductions performed for postdatism, for the early detection of multifetal gestations, for detection of placental implantation abnormalities and for the antenatal diagnosis of congenital anomalies.

There is good evidence to support the recommendation that the sensitivity of the ultrasound screening in detecting fetal malformations in low risk pregnancies cannot be established with precision it will continue to be decided on a local level and varies in different centers with different level of operators training and financial resources. Sonography for fetal biometry and when precise estimation of gestational age is required (in cases such as planning a caesarean delivery), should be performed in the first trimester or as early in pregnancy as feasible. 18-20 weeks is the traditional and appropriate time to perform a targeted scan. This ultrasound study allows a detailed review of fetal anatomy and is early enough so that amniocentesis or other diagnostic procedures can be performed prior to fetal viability.

The genetic sonogram is a targeted study with special emphasis on ultrasonographic markers that may indicate aneuploidy. Targeted ultrasonography at 18-20 weeks allows the couple to consider all of their options and allows for appropriate referral and counseling. However some malformations are not easily visualized at this period. Hydrocephalus or bowel atresia's may develop after this period and may not be demonstrable until after 24 week's gestation while the optimal time for fetal echocardiography is probably somewhat later (20-22 weeks).

By whom: Antenatal sonography is performed in different medical centers, doctor's offices, hospitals, by physicians of varying levels of experience or by technicians. If a physician is unable to document formal residency, fellowship, or other postgraduate training, he or she must have completed 100 hr of American Medical Association category 1 continuing medical education in diagnostic ultrasound, with evidence of involvement at least 500 diagnostic examinations under the supervision of a qualified physician. The experience of the obstetrician clinician with sonography must begin with detailed knowledge regarding fetal cross sectional anatomy. It is important for the clinician to know his or her limits with regard to the use of ultrasound. Limitations of obstetrical ultrasonography should be briefly reviewed with patients prior to the initiation of the procedure. Some major malformations are easily detectable whereas other malformations present subtle ultrasound images, and may not be diagnosable in the midtrimester. Ultrasound is used not only for diagnosis but as a tool for the management of a complicated pregnancy and for this reason the perinatologist is perfectly the right doctor to provide sonographic diagnosis and plan the management of a high risk pregnancy.

Conclusion: The issue of routine sonography for low risk pregnant women continues to be contentions even though, randomized trials have not been able to demonstrate a clear benefit. Although great progress is being made in the first trimester diagnoses of congenital anomalies, most targeted studies are performed at 18-20 weeks of gestation. The highest rates of detection of congenital anomalies are seen in tertiary care settings such as a university medical center. In high risk cases a consulting perinatologist is commonly the physician most likely to integrate the ultrasound findings.

K-7
The role of hysteroscopy in female infertility management

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Implantation is an important factor that was influenced with the embryo and endometrium dialogue. The uterine evaluation before any assisted reproductive technique should do as a routine procedure. Hysterosalpingography is the first method of uterine abnormality evaluation. But different researches have shown the false negative result of HSG in uterine abnormalities in 18.4%. Hysteroscopy is the gold standard procedure for uterine cavity exploration through direct visualization in patients with recurrent implantation failure. It appears that more than 1/3 of the patients interpreted as normal following HSG are found to have a uterine abnormality after diagnostic hysteroscopy, which might be a significant cause of reproductive failure.

Polyps are the most common pathological lesions in infertile women especially in unexplained infertility. The possible role of these polyps in infertility is yet unclear but surgical removal of all endometrial polyps among infertile women is crucial. Removal of polyps may enhance reproductive outcome between 43-80%. The mullerain abnormalities in the uterus such as septate, subseptate, arcuate and bicornate are common findings in the hysteroscopy of repeated IVF failure

patients with previous normal HSG. Although WHO is recommended the office hysteroscopy when clinical or complementary exams such as ultrasound and HSG suggest intrauterine abnormality or after IVF failure but it is a routine procedure before the first ART cycles due to enhancing fertility. Implantation improvement after hysteroscopy could be related not only to treating uterine cavity lesions but also may be affected by cervical canal dilation and evaluation of the direction of the cervical canal for easy embryo transfer, assessment of interior of the uterine cavity and shape abnormality. Moreover, the uterine instrumentation cause endometrial injury and stimulates inflammatory reactions to growth factors and may improve the pregnancy rate by near 2 folds (32-44% vs. 21-26%). Although the position of hysteroscopy in infertility management is unclear but diagnostic hysteroscopy is a valuable test and should be advised routinely as part of patients' investigations before IVF/ET.

K-8
Different clinical presentations of endometriosis

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Endometriosis is the third leading cause of gynecologic hospitalization in the United States. Endometriosis can develop between 10 and 60 years of age. The average age of diagnosis is 27 years. This disease impacts both a woman's physical and mental wellbeing. This impact is often compounded by the frequent delay of 6 years or more from the onset of symptoms to a confirmed diagnosis, which may. Because there is no good noninvasive test for endometriosis, there is often a significant delay in diagnosis of this disease. Among women who seek tubal ligation, the prevalence of endometriosis appears to range from 2-18%, whereas within infertile populations it has been reported to be as high as 50%.
No serum marker has been found to diagnose endometriosis with adequate sensitivity and specificity. There has been a recent focus on the presence of nerve fibers in the eutopic endometrium of patients with endometriosis. There is a wide spectrum of symptom severity, clinical presentation and the stage of endometriosis. Laparoscopy is the gold standard for diagnosis of endometriosis but in the hands of expert laparoscopic surgeon 6-10% of endometriosis is missed. Some patients with minimal disease have debilitating pain, whereas other women with severe stage III to IV disease are asymptomatic. Women with mild to moderate endometriosis have a higher incidence of endocrine abnormalities, anovulation, corpusluteum insufficiency, hyperprolactinemia, luteinized unruptured follicle syndrome, and spontaneous abortions. Sonography was found to have up to 84% sensitivity and 90% specificity for the detection of endometriomas, confirmed by surgery and histopathology. The goal of surgical treatment is to remove visible areas of endometriosis and restore normal anatomy by lysis of adhesions.

K-9
First and second trimester screening for aneuploidy

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Over the last two decades, risk assessment for aneuploidy has been refined to the point that maternal age alone is no longer considered adequate in determining the risk of having a chromosomally abnormal offspring. Obstetric sonography, in conjunction with serum analysis, has become a powerful tool in the assessment of risk for aneuploidy, in both the first and the second trimester. In the mid trimester the diverse sonographic patterns seen in the different aneuploidies allows clinicians to guide patients to a presumptive diagnosis. The information obtained noninvasively helps the expectant couple to weigh the risks of invasive testing against the probability of having a child with an abnormality. The goal of screening is the detection of a greater number of karyotypically abnormal fetuses with fewer invasive procedures and subsequently the loss of fewer normal fetuses. First and second trimester markers will be discussed.

K-10
Religion, law and ethics: Ethical and anthropological reflection on assisted reproduction

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This paper seeks to have a close look at what happens with cosmological phenomena, economic values, conflicting moralities and kinship principles when they meet clinical practices, legislations and regulations pertaining to reproductive technologies, including gamete and embryo donation as well as surrogacy arrangements. Mainly, based on my extensive ethnographic research on assisted reproductive technologies in Iran, which includes an examination of the normative arguments, this paper attempts to explore what moral, theological and legal reasoning underlie the concepts of kinship and reproduction that move people when they turn to- or refrain from- certain technologies

of assisted conception? And how are they contested and negotiated? I acknowledge the importance of contextual understanding of moral concepts, arguments and reasoning involved in the application of reproductive technologies as well as the interplay between religious, moral and legal ideas and institutions, and the place of this interplay in contemporary debates surrounding human reproduction and reproductive health. I view reproduction as a process through which the foundational structures and perceptions of a society and its dynamics are reproduced and contested rather than a sexual act or as simply the combination of male and female reproductive substances. Reflections offered in this article are based on my doctoral research project "Assisted Reproductive Technologies in Iran from an Anthropological Perspective: Legal and Jurisprudential Responses and Social Dynamics" that has examined the Iranian and contemporary Shia legal debates and discussions on technologies of assisted conception and has looked at the regulations and implementation of these technologies in Iran.

K-11
Antioxidants and their role in male infertility

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Male infertility constitutes about 50% of cases of infertility. In nearly half of these patients no specific etiological cause could be found. In recent decades much attention has been paid to the role of overproduction of free radicals in semen and oxidative stress on different sperm functions. Also sperm chromatin damage which could be a result of oxidative stress or other known factors such as varicocele or smoking has been implicated in etiology of male infertility. With respect to these new findings removal of free radicals and elimination of factors that could potentially induce sperm chromatin damage became more important. With regard to the above mentioned findings, during the past few years a variety of different antioxidant medications and even foods rich in antioxidant compounds had been tried to treat these patients. Many studies and reports evaluated the effect of these compounds on improving sperm parameters and improving pregnancy rate. Some of these studies showed the positive effect of antioxidants on fertility potentials while other observed no significant effects. Also there is a still controversy on the best type of antioxidant and even the optimal dose of these compounds in the treatment of male infertility, which needs more clinical trials and further studies. In conclusion it seems that antioxidants have an important role on improving male infertility and its use in patients with idiopathic male infertility is strangle advised, yet more study on this subject is necessary to elucidate the best compound and the optimal doses and duration of these medications.

K-12
The sperm aging: Is it affecting ART outcomes?

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Sperm aging is usually the concerns of many basic and clinical studies recently. This topic can be considered from three different perspectives. Most of studies are focused on the paternal age on quality and quantity of sperm and also its consequences on the older men fertility, in vitro fertilization outcomes and the health status of infants born from these parents. Its second aspect is sperm aging in time interval between spermiation and ejaculation. Based on the tracing of radiolabeled molecules, usual duration of sperm journey is approximately two weeks, but it is highly affected by time interval between ejaculations. The third aspect of sperm aging is lapse of time from ejaculation. Human sperm exposed to a physicochemical condition following ejaculation in a container that are very different from in vivo condition of male and female genital tracts. These physicochemical changes can lead to deleterious consequences on sperm structure and function. Several studies on aged men in comparison younger ones showed that the spermogram parameters decreased significantly in older men. Sperm chromatin integrity and DNA fragmentations, as well as aneuploidy abnormalities significantly increased with men's age.
In infertile men with oligoasthenoteratozoospermia (OAT) too long intervals between ejaculations lead to decrease sperm quality. However multiple ejaculations in short interval in these patients significantly increased the sperm parameters and its chromatin integrity. There is negative correlation between the ejaculation to analysis, processing and insemination intervals. So that sperm parameters significantly declined during in vitro storage of sperm especially following processing and elimination of seminal plasma.Therefore, ignoring of the sperm aging in relation to human fertility, especially assisted reproductive technologies can have serious influences on natural fertility, IVF outcomes and the health of associated offspring.

K-13
New markers in male fertility evaluations

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It is obvious that the assessments of spermatogenesis, semen and endocrine analysis are critical in the evaluation of the infertile and subfertile couples. But, it seems that currently used methods are inadequate to correctly predict sperm fertility potential and do not provide sufficient information for diagnosing and treatment of some clinical infertility situations. Conversely, the hidden and unclear sperm abnormalities impairing the reproductive success of sperm and egg interaction often remain undiagnosed and in these cases of unexplained infertility, there are no clear reasons for the condition. So, many laboratory tests have been developed in order to evaluate the structure and function of human spermatozoa.

In recent years, researches have focused on identifying reliable markers of fertility at the genomic, proteomic, biochemical, and immunocytochemical levels. The use of fluorescent markers to assess the acrosomal status, the use of vital staining for mitochondrial activity and energy metabolism and the use of particular fluorochromes and cytochemical dyes to detect altered sperm chromatin or DNA along existing functional tests like the hypo-osmotic swelling test and the hemi-zona assay are the most useful assessments of spermatozoa. Additionally, an association between infertility and seminal oxidative stress has been suggested. Excessive ROS production damages the sperm membrane, reduces motility, induces permanent DNA damage and it is closely associated with apoptosis. ROS production can be directly monitored by a luminol or a lucigenin-based chemilluminescence assay and the apoptosis can be detected by several molecular and immunocytochemical methods. In many cases of male infertility, the cause is genetically in origin.

Thus, in the context of reproductive research, genetic defects in gametogenesis are being extensively studied and many important genes in sperm biology have detected so far. Finally, the proteomics or comprehensive study of proteins with their particular structural and functional aspects, have allowed the identification of different proteins in semen and spermatozoa that are responsible for the regulation of normal/defective sperm functions. Presently, numerous proteomics techniques, such as two-dimensional (2D) polyacrylamide gel electrophoresis, and mass spectrometry are widely used to identify sperm-specific proteins. These assays help us to understand different functional aspects of sperm proteins which are important in motility, capacitation, acrosomal reaction, fertilization, chromatin remodeling and posttranslational modifications.

K-14
Exogenous HSPA8 prolongs sperm survival by enhancing membrane fluidity in a cholesterol-dependent manner

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Introduction: In many female species, sperm viability is maintained by storing spermatozoa in the reproductive tract prior to fertilization via the temporary attachment of sperm heads to the apical oviduct epithelial cells (OECs). Despite its importance in assisted reproduction, the mechanisms involved in the prolongation of sperm survival *in vivo* are not fully understood. It has been reported that the presence of sperm in the female oviduct induces the expression of the constitutive member of the 70 kDa heat shock protein family, HSPA8, in the oviduct as an exogenous protein and we have shown that exogenous recombinant HSPA8 enhances survival and membrane fluidity of boar spermatozoa in vitro. The aim of this study is to provide insight into the capacity of exogenous HSPA8 to extend sperm survival and mechanism of HSPA8-sperm interactions.

Materials and Methods: The localization of fluorescently conjugated exogenous HSPA8 ($ATTO^{488}$-HSPA8) following incubation with boar spermatozoa (0.5 µg/ml, 15 min, room temperature) was determined by confocal microscopy. Sperm viability (membrane integrity) was assessed using SYBR-14/propidium iodide. Membrane fluidity (D values and Recovery %) of acrosomal and posacrosomal domains of live cells was measured using fluorescence recovery after photobleaching (FRAP). The influence of membrane cholesterol on the ability of HSPA8 to modulate sperm membrane fluidity was examined by depleting membrane cholesterol using different concentrations of cyclodextrin (0, 2, 4, 8 mM, 30 min) and replenishing cholesterol using cyclodextrin-cholesterol complexes. Data are expressed as mean±SEM.

Results: $ATTO^{488}$-HSPA8 binding was localized to the acrosomal sperm membrane and HSPA8 had no effect on the viability of cholesterol-depleted spermatozoa. Cholesterol removal reduced D values for the acrosome (46±3 vs. 29±2, $p<0.01$) and postacrosome (34±6 vs. 19±4, $p<0.005$). R% values were also significantly lower. Reloading cholesterol restored membrane fluidity and the ability of HSPA8 to increase viability.

Conclusion: Spermatozoa are devoid of protein synthesis apparatus and incapable of *de novo* protein synthesis under stressful conditions. These findings suggest that exogenous heat shock proteins can maintain the integrity of biologic membranes and act as 'rapid response' extracellular cytoprotectors in a cholesterol-dependent manner.

K-15
Pluripotent stem cells and regenerative medicine

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Introduction: Pluripotent stem cells have been defined as those with the ability to give rise to all tissue types from the three embryonic germ layers (ectoderm, mesoderm, and endoderm) and the capacity for indefinite self-renewal if cultured in appropriate conditions. Two different cell types, embryonic stem cells (hESC) and induced pluripotent stem (iPS) cells; have been demonstrated to be pluripotent.

Human embryonic stem cells: Human embryonic stem cells (hESC) were first described in 1998 by Thomson and are derived from human embryos, mainly from the Inner Cell Mass (ICM) of the blastocyst, at day 5-7 of development. Other options for hESC derivation include early embryos, morulae and single cells. Embryos donated for research by couples undergoing In Vitro Fertilization treatment constitute the main source for hESC derivation. The methodology may vary among the different groups and no standardized protocol for derivation has been described. To date, more than one thousand hESC lines have been derived worldwide and even though an international registry is still lacking, more than 600 European and international hESC lines have been registered at the human Embryonic Stem Cell registry, a project funded by the FP6 work programmer of the European Commission.

Induced pluripotent stem cells (iPS): In 2006, Yamanaka and co-workers described the possibility of reprogramming the nucleus of mouse somatic cells into a pluripotent state by the ectopic expression of a defined set of genes. These cells were called induced pluripotent stem cells (iPS). A year later, 2 different reports described the methodology to generate human iPS by retroviral transduction of 4 different sets of genes (Oct_4, Sox_2, Klf_4 and c-Myc and Oct_4, Sox_2, Nanog and Lin28). These cells exhibit most of the characteristics seen in hESC, such as morphology, proliferation ability and pluripotency. A number of publications have demonstrated that somatic cells from different origins can be reprogrammed to iPS (fibroblasts, keratinocytes, liver cells, neural stem cells, cord blood cells, etc.) with the use of a limited number of transcription factors. Also, the mode of delivery of such factors has been modified to achieve safe reprogramming.

Clinical translation of pluripotent stem cells: There are a number of major drawbacks that need to be resolved to ensure the safe application for therapy of pluripotent stem cells, including hESC and iPS. One of the major issues to be solved is to determine which cells have to be transplanted, and specifically at what stage of differentiation. Also, when transplanting into solid organs, the 3D support for transplantation and integration also has to be considered. Differentiation protocols have to be optimized in order to produce pure populations. Large scale and GMP production of cells are required. Similar to organ transplantation, immune rejection should also to be considered. hESC banks that include the most frequent haplotypes have to be put in place. iPS generation for specific patients or from cell types previously banked, such as cord blood cells, may solve the problem in reprogrammed cells. A clinical trial involving the use of hESC derived Retinal Pigmented Epithelium for Macular degeneration is currently in place in the US and UK and the same protocol have been approved with iPS cells in Japan. The field of stem cell research and regenerative medicine holds a promising future for the treatment of degenerative diseases.

K-16
Next steps towards the transplantable artificial ovary

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In recent years, advanced chemo/ radio therapeutic treatments have led to high survival rates in cancer patients, giving rise to new issues for cancer survivors. Indeed, one major concern is future fertility in these women, since they may face premature ovarian failure. For this reason, different strategies have been proposed to preserve their fertility. When gonadotoxic treatment cannot be delayed, ovarian tissue cryobanking appears to be the most promising way of preserving a patient's fertility. Moreover, this is the sole means of safeguarding fertility in prepubertal girls. Auto transplantation is the only option able to reestablish ovarian function from cryopreserved ovarian tissue in cancer survivors at present. So far, this technique has led to successful ovarian function restoration and up to 40 pregnancies in a number of centers around the world. However, there is a legitimate concern regarding the possible presence of malignant cells in frozen-thawed fragments, which could provoke a recurrence of the primary disease after re-implantation. Although many types of cancer never metastasise to the ovaries, leukaemia is systemic in nature and poses a greater threat to the patient, while breast cancer and some types of lymphoma are classed as moderate risk.

For these patients, a safer alternative could be grafting of isolated preantral follicles, as these structures are enclosed in a basement membrane that prevents direct contact between follicular cells and capillaries, white blood cells, and nerve processes. Since ovarian cells (OCs) are essential for follicle development and neovascularization, autologous OCs should be grafted together with isolated follicles. To replace the original ovarian structure, a transplantable artificial ovary (TAO) should be created in order to encapsulate and protect the isolated follicles and OCs. As in case of a natural ovary, the main goal of the TAO is to offer an

environment that allows follicle survival and development. Therefore, a TAO should maintain the original structure of follicles, ensure proper communication between follicles and OCs, and preserve their interaction with the extracellular matrix and supply factors involved in follicular survival and development. In other words, the TAO should spatially and temporally mimic the ECM. In order to do so, it should include some design parameters, such as physical support of follicles, porosity, bioactivity, vascularization, interaction with cells, and biodegradability, which are all interconnected and influence each other.

K-17
Mitochondria and oocyte maturation

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Mitochondria are critical organelles within the cell and has important role in the oocyte development. The number and distribution of mitochondria, and energy (ATP) production are critical factors that influence not only on the maturation and development of the oocyte but also on its fertilization, and subsequent embryo development. Structural and metabolic mitochondrial defects are associated with failures in oocyte maturation and abnormal development or arrest of embryos. Mitochondrial content could affect the fertilization potential of oocyte. If mitochondrial DNA content of oocyte be lower than threshold, it's more prone to failed maturation and showed reduced fertilization rates. Dysfunction of oocyte mitochondria may occur without detectable morphological abnormalities.

K-18
Organelle morphodynamics in human mature oocytes after cryopreservation. Ultra structural analysis at different time intervals during thawing

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Introduction: During freeze-thawing, the human metaphase II (MII) oocyte is exposed to a variety of physical and chemical conditions that may endanger its competence to fertilization and even its mere survival. In this study we evaluated presence and amount of: a) ooplasmic vacuolization, b) organelle-specific associations such as mitochondria-smooth endoplasmic reticulum (M-SER) aggregates and mitochondria-vesicle (MV) complexes, and c) cortical granules (CGs).
Materials and Methods: MII oocytes were subjected to slow freezing through two-step propanediol (PrOH) dehydration with 0.75-1.5 mol/l PrOH and 0.2 mol/l sucrose and examined by light and transmission electron microscopy (TEM) at different time intervals during thawing. Cryopreserved oocytes were fixed after being transferred in 1.0 mol/l PrOH and 0.3 mol/l sucrose (group A, n=15), 0.5 mol/l PrOH and 0.3 mol/l sucrose (group B, n=15) and 0.3 mol/l sucrose (group C, n=15). Fresh MII oocytes (n=15) were used as controls.
Results: Morphometric and TEM analysis revealed that vacuoles were only occasionally detected in the ooplasm of fresh controls. Conversely, vacuoles were numerous in the cryopreserved oocytes of group A and appeared to reach an even larger number in group B oocytes. M-SER aggregates, large and abundant in the ooplasm of fresh controls, significantly decreased in number following freezing, particularly in the oocytes belonging to groups A and B. MV complexes were instead small and scarce in fresh control oocytes but augmented after freezing, being especially abundant in the oocytes belonging to group B. Vacuoles and MV complexes both diminished in the oocytes belonging to group C, whereas M-SER aggregates increased in number. CGs was scarce in all cryopreserved oocytes in respect to those found in fresh controls and gradually diminished as thawing progressed.
Conclusion: This study proves that vacuoles, generally regarded as markers of oocyte cryodamage during slow cooling, may form during freezing, but become numerous during thawing, particularly when the lowest concentration of PrOH is reached. Significant variations in the number of M-SER aggregates and MV complexes occurred during the freeze-thawing, suggesting a dynamic process of transition between these two forms of organelle associations. This study also evidences that a premature CG exocytosis progressively occurs during the whole freeze-thawing procedure. It seems also worth noting that all systems of ooplasmic membranes appear significantly concerned by freeze-thawing but, except for CGs, their alterations seem to undergo a partial or, more rarely, an almost complete recovery at the end of the thawing process.

K-19
Ultrastructural markers of aging in human oocytes

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Introduction: The delay of childbearing contributes to the increasing proportion of subfertile couples necessitating assisted reproduction technology (ART) procedures. Subfertility relates with decay in oocyte quality due to reproductive aging, indeed maternal aging impairs reproductive potential. Prolonged culture, also called "in vitro aging" may also impair oocyte competence. Ultra structural oocyte quality greatly affects ART outcome that also depends on to specific morphological parameters. In this report, we account for the ultrastructural markers of aging, in oocytes from over-35 years old women underwent to ART procedures, enrolled in this study after informed consent.
Materials and Methods: We studied MII oocytes from women under 35 and over 35 years old, fixed at pick up or after 24 hr culture. Ultrastructural and morphometric evaluations were performed.
Results: Significant increasing of vacuoles, decreasing of mitochondria-smooth endoplasmic reticulum aggregates, increasing of mitochondria-vesicle complexes density, decreasing of cortical granules and microvilli, increasing of zona pellucida density and thickness, characterized oocytes from aged women. These changes were more evident in the oocytes submitted to prolonged culture.
Conclusion: These changes may be assumed as ultra-structural markers of oocyte aging. It was also demonstrated that oocytes from younger women are less sensitive to prolonged culture (in vitro aging) than the oocytes from aged women.

K-20
Manipulation of human oocytes and embryos to diagnose and treat from point of embryologist view

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With the birth of the first human baby by IVF techniques in 1978 AD, this area of medicine is in very rapid advances in the diagnosis and treatment of infertility. An experienced clinical embryologist using advanced facilities can perform various manipulations on oocytes and embryos. Chromosomal and genetic analysis of oocytes and embryos biopsied cells could be helpful in the diagnosis of many diseases. To avoid false reports because of mosaicism, geneticists suggest two cells are removed, but removing more than one cell can cause more damage to the embryo. There are much debates about the number of cells, the embryonic stage and the technique of biopsy. Quality control after biopsy is most important issues in this regard. Naturally before implantation, embryo hatched from zona pellucid, which in some cases, such as aging and freezing, this hatching does not happen. So assisted hatching can be helpful in these cases. Removal of degenerated cells from fresh embryos or embryos after thawing in some cases, may be helpful to keep a better growth of the embryo. Transferring the nucleus, cytoplasm and mitochondria of healthy oocyte in the oocyte case is currently being done in some countries. It should be noted that many other manipulations such as human cloning need the legal and ethical permission.

K-21
Foreseeing the fate of the embryo- Advances and limitations of time-lapse technology

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Embryo evaluation is a crucial part of the infertility treatment. It supports the selection of the right embryo from the cohort for transfer if performed properly. A good scheme for embryo assessment assists elective single embryo transfer, regardless if a clinic utilizes fresh and cryopreserved transfers or follows the "freeze all" strategy. Moreover, a proper evaluation system coupled with proper embryo culture conditions and manipulation techniques can have an effect on stimulation protocols in favor of mild approaches, resulting in lower hormonal load, fewer but higher quality embryos, and, consequently, higher embryo utilization.
Embryo evaluation techniques have been developed from the advent of mammalian embryology. These were based on static evaluation of the morphology at certain time-points during in vitro culture. Scores were established to evaluate certain morphological features including pronuclear pattern, zona pellucida, blastomere number at certain time-points, extent of fragmentation, cytoplasmic appearance, and blastocyst morphology. For this, embryos had to be removed from the incubator and checked. How often? Preferably not at all, but to get any information about embryo quality during in vitro development one needs to make compromises, so frequency for embryo checkups generally range from 1-5 times during the 5 days of culture.
One of the advances of using time-lapse techniques is to follow up embryo development and to obtain information on morphology. This information is provided continuously every 5-20 min, not just at

distinct time-points of the day but at any time-points of the day. Techniques now available make it possible to perform embryo evaluation while embryos are inside of the incubator, thus reducing handling stress. During a course of routine time-lapse examination, hundreds of images are made and saved, archived in digital format, enabling another fundamentally important expectation of the scientific society: proper documentation and quality control of the laboratory phase, right at its heart: inside of the incubator. Apart from quality control there is another everyday use of the digital imaging, and that is learning, teaching and communication. The listed possibilities alone justify the use of time-lapse technology in the embryology lab. However, the exponentially increased number of information provided by time-lapse technology has put a question mark onto the reliability of the well-established morphological scorings. Moreover, a new set of information became available, as time-lapse enables us to calculate with the length of interphases and the duration and synchrony of cytokinesis, and use this information when quantifying embryo quality. How does time-lapse technology change how see and grade embryos?

What do we learn from continuous embryo follow-up?

Pronuclear scoring involves the assessment of the number and relative position of the nucleolar precursor bodies (NPBs) which are established in the pronuclei. Any inequality in the distribution of the NPBs within the pronuclei is considered to be abnormal, but time-lapse studies reviled, that NPBs move around inside of the pronuclei, and can produce up to 2 score difference within 2 hr, making their traditional, "static evaluation" and its value questionable in the present format. Morphologically, early cleaving embryos have been regarded as higher quality ones. However, early cleavage has lost its classic meaning in the time-lapse environment. At checking time-lapse recordings of embryos we are looking for timeframes for cleavages, as too early cleavage can equally be an unfavorable sign of embryo quality as cleaving too late. Such simple questions as cell number at certain time-points get also questioned, when it became possible to follow the cleavage pattern of the actual embryo. A morphologically sound five cell stage embryo can reach the five cell stage by normal but also abnormal cleavage paths. A five cell stage embryo can be the result if the first cytokinesis produced 3 blastomeres, 2 of which cleaved further. After a normal first cytokinesis, one blastomere can cleave to 2 cells, while the second one may cleave to 3 daughter cells, resulting, again, in a normal looking but abnormal five cell stage embryo. Though the morphological evaluation may reveal same score for the given examples, their potential to implant and to develop to a healthy offspring differ significantly.

Fragmentation has also been observed as highly dynamic process with fragments being continuously rearranging around the blastomeres or being reabsorbed during the course of in vitro development. For this reason, static evaluation of fragmentation might not be absolutely correct. A further example for the dynamic nature of morphology is the fact that blastocysts pulsate: they expand and collapse continuously. An expanded blastocyst may collapse within a short time, whereas her blastocyst score would change, while her quality would not.

Time-lapse projects also provide insight into the timings of the cell cycle. Embryos are supposed to cleave within a definite time-frame. Which are the most important events, and are they in correlation with blastocyst formation of pregnancy? Recent studies have revealed that cleavages up until the 4 cell stage are more predictive to the chance to reach the blastocyst stage, while events prone to happen after the onset of the genomic activation seem to provide information that is relevant to pregnancy. According to our group, morphokinetics in itself is not sufficient for proper embryo evaluation; it has to be applied in combination with static morphology. Nevertheless, time-lapse is needed to qualify static morphology properly. Focusing onto the importance of morphokinetics purely, our group sees its role in supporting de-selection. De-selection in this content means embryos performing irregular cleavage like directly cleaving from one cell to three cell stages are ranked back in the cohort with the note of lower chance for implantation.

Up to date there are numerous equipment available that can host live cells and follow up their development while maintaining and supporting close to physiological environment around the cells. The most classical type of equipment designed for live cell imaging is a regular inverted microscope with a plastic cask built around it. In the plastic box temperature, humidity and gas concentration can be adjusted up to certain precisity, with uneven distribution. An alternative solution is to apply a small incubation box (stage-top incubator) onto the microscopic stage. This type of equipment, available from all major microscope manufacturers and also home-made editions do not satisfy the delicate needs of the embryo for a precise and stable environment. However, with the use of an environmental chamber in combination with a stage-top incubator made it possible to follow up human embryo development until the 4 cell stage. Further developments included the inclusion of a proper, automated inverted microscope into a regular incubator. This setup needs a quasi-robotic system that moves the embryos into the field of view. Besides the adequate optical output, the efficacy of the earlier versions were hampered by the complicated inner structure conveying heat accumulation due to friction, VOC due to lubrication, sheer stress due to movement,

and electromagnetic field due to electricity needed inside of the culturing space. Besides, their use in the routine was cumbersome. The structure of these devices has been refined, and dedicated equipment has been developed, keeping the same principles and risks, serving the routine in human ART. Another approach has been presented to everyday lab use, in 2008; this follows the principle of providing not just embryo monitoring, but a cell-stress free culturing environment. In this setup the inverted microscope has been compacted to fit even a dozen into a regular size incubator.

These microscopes are completely sealed, making it possible to use in 100% relative humidified environment, and bear a custom designed, high resolution, super wide-field optics, which can see and individually identify up to 16 embryos of a patient in one field of view, without the need to move anything. This way all the possible stress factors (sheer stress, heat, VOC, electromagnetic field) are eliminated. Moreover, embryos can be cultured in groups, which give a clear benefit over single embryo culture. Today close to 600 clinics are using one of the two time-lapse solutions, and have provided evidence that this type of embryo follow-up is needed even in the everyday routine, for embryo evaluation, quality control and service.

K-22
Novel applications of somatic cell nuclear transfer in animals and human

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From historical point of view, somatic cell nuclear transfer (SCNT) or cloning was first introduced to answer a fundamental scientific question which was: "Do all the cells of the body contain the same genetic information"? However, today through the technique of SCNT not only we can answer many of the scientific questions, especially in the field of epigenetics but also SCNT have different applications including saving of endangered species, production of elite animals, in vitro production of human diseases models and production of human organ in reconstructed animals. However, the other question which has gained much attention in the scientific community is that: May SCNT technique dominates the well-established technique of induce pluripotent stem cells (iPS) for production of human stem cell for clinical applications? Therefore, this presentation hope to expand over different applications of SCNT, especially in the field of human organ production and clinical application of SCNT in production of therapeutic human stem cells.

K-23
Stem cells for infertility treatment

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Infertility affects an estimate of 20% of Iranian couples that is higher compare to other countries. Current infertility treatments are limited to techniques such as in vitro fertilization (IVF) that have serious side effects related to drugs and low success rate which cannot cure many infertility types. Of new therapies, stem cells have opened new window for infertility treatments. In this regard, pluripotent stem cells (PSCs) are promising to generate an unlimited source of germ cells and gametes for infertile couples. In this purpose, Hayashi *et al* reported production of both male and female gametes (sperm and oocyte) from mouse embryonic stem cells (ESC) and induced Pluripotent stem cells (iPSC). The in vitro produced gametes had successful fertilization and lead to healthy and fertile offspring. More recently, report of germ cell differentiation of iPSCs from azoospermia men bring us a lot of hope for infertility treatment. As parallel sources, germ line stem have been considered. In this regard recently introduced oogonial stem cells (OSC) are promising for female infertility treatment. Besides, testis derived spermatogonial stem cells (SSCs) have been differentiated to functional sperms in the laboratory. All these reports bring us lots of hope to cure the infertility in future by stem cell technology.

K-24
Spermatogonial stem cells

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The process of spermatogenesis is initiated and maintained by a rare population of single spermatogonial stem cells (SSCs). The SSCs are attached to the basement membrane of the seminiferous tubules and are characterized by typical morphological criteria. SSCs are the important starting point as part of a robust stem cell system of the testis, involved in spermatogenesis and reproduction. The isolation and cultivation of human SSCs significantly contributes to the increasing knowledge of human germ and stem cell biology.

Although still a difficult task, the newly established enrichment and in vitro propagation of spermatogonia that carry the male genome from generation to generation provides an important step for future transplantation and restoration of fertility in the clinic.