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.

Award Winners

First winners *(Alphabetic order)*

A-1a
Artificial seminal fluid preserves human sperm quality in vitrification program: An electron microscopy study

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Introduction: This study compared the effects of three different media on human sperm parameters, and ultrastructure of spermatozoa using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after vitirification. These media were artificial seminal fluid (ASF), seminal fluid (SF) and human tubal fluid (HTF)-sucrose.
Materials and Methods: 30 normal ejaculates were processed with swim-up technique and sperm suspensions were divided in four aliquots: 1) fresh sample (control); 2) vitrification in HTF supplemented with 0.25 mol sucrose, as routine procedure; 3) vitrification with patients' SF; and 4) vitrification in ASF. After warming, sperm parameters of motility, viability and morphology were analyzed using WHO criteria. Also, sperm pellets were fixed in 2.5% gluthardehyde and processed for SEM and TEM observations.
Results: Sperm parameters in all cryo-groups were reduced when compared with control samples ($p<0.0001$). Briefly, sperm grade A motility, viability and normal morphology were significantly higher in ASF than HTF group. After cryopreservation, deep invagination in cytoplasm, mechanically weak point sites, rough membrane surface and looped tails were observed in SEM evaluation. The looped tails were more severe in SF and HTF cryo-groups. In TEM evaluation, acrosome damage, plasma membrane loss, chromatin vacuolation, and disruption of mitochondria arrangement and structure were observed in all cryo-groups. Degradation of cells was also observed, especially in HTF cryo-group.
Conclusion: Vitrification of human spermatozoa with ASF can effectively preserve the quality of motility in comparison with routine procedure. This ASF medium formulated according to SF that is a natural medium for sperm preservation, lacking artificial components, such as sucrose. With this design, any osmotic shock can be deleted before and after freezing. Also, this study confirmed that SF in normal ejaculates can act as cryoprotectants.
Key words: *Artificial seminal fluid, Seminal fluid, Vitrification, Human spermatozoa.*

A-1b
Proteomic profile of human endometrium in normal women compare to polycystic ovarian syndrome patients

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Introduction: Endometrial receptivity seems to be the major limiting factor for the success of pregnancy in polycystic ovarian syndrome (PCOS). PCOS is the most common cause of female infertility affecting approximately 5-10% of premenopausal women. The aim was to identify the changes in whole proteins between PCOS and normal endometrium.
Materials and Methods: In this study, for the first time, a 2-DE based proteomic approach coupled with mass spectrometry was used to identify the changes in whole proteins between PCOS and normal endometrium. We analyzed proteome of endometrium during proliferative (n=6) and luteal phases (n=6) from healthy women and PCOS patients (n=6). To validate this investigation western blot and quantitative real time PCR were performed.
Results: About 802±10 protein spots reproducible detected on gels, 170 protein spots showed different intensities between PCOS, proliferative and luteal endometrium. Mass spectrometry analysis detected 70 proteins out of 170 spots. The expression of Annexin A5 (ANXA5), 14-3-3 protein, Serpin A1, Cathepsin D proteins was validated by western blot. In addition, the gene expression profile of these proteins was confirmed by real time Q-PCR. The results obtained in the western blot and real time PCR followed a similar regulation of proteomic analysis.
Conclusion: This study provides the first insight into the global protein expression in the endometrium of PCOS patients in compare to normal women which affect endometrial receptivity. Mass spectrometry analysis of differentially expressed proteins between PCOS and normal endometrium resulted in identification of 70 proteins involved in cellular metabolism, oxidative stress, apoptosis and

immunological process. Each of this process absolutely demonstrates an important role in fecundity and fecund ability. So the present study may reveal the cause of various endometrial aberrations in women with PCOS. Our investigation also provides novel information on differential expression of several proteins in secretory phase endometrium compared to proliferative in healthy fertile women. It will create a basis to establish the functional networks that operate as an inducer of the endometrial receptivity.

Key words: *Endometrium, Proteomics, PCOS, Proliferative phase, Secretory phase.*

A-1c

Activation of Toll-like receptor 3 reduces actin polymerization and adhesion molecule expression in endometrial cells, a potential mechanism for viral-induced implantation failure

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Introduction: Embryonic implantation is a critical event which leads to successful pregnancy. This requires communication between the endometrium (mother) and the embryo. It is well-documented that the presence of an infection at the time of implantation can lead to implantation failure. The female reproductive tract recognizes invading microorganisms through the innate pathogen recognition receptors (PRRs) such as the Toll-like receptors (TLRs). To date, 10 members of TLR family have been recognized in human (TLR1 to 10). Our earlier results have demonstrated that the stimulation of TLR 2/6 and 5 in the maternal tract can reduce implantation chances *in vivo* and *in vitro*. In the current investigation, we determined whether the activation of TLR 3 could affect the binding of trophoblast cells to endometrial cells. We also assessed if TLR 3 activation could affect actin polymerization or the expression of adhesion molecules such as β3 and CD98 in endometrial cells, since these changes could represent the molecular mechanism responsible for TLR 3 suppression of trophoblast cells adhesion to endometrial cells.

Materials and Methods: An in vitro assay was developed using RL95-2 (an endometrial cell line) and JAr (a trophoblast cell line) cells. Initially, the percentage of attached JAr spheroids to RL95-2 was measured in response to TLR 3 activation. Next, actin polymerization in RL95-2 cells was assessed in response to TLR 2/6, 3 and 5 activation. Phalloidin was used to assess the mean fluorescence intensity of F-actin by flow cytometry or confocal microscopy. Secondly, the influence of TLR 2/6, 3 and 5 activation on the expression of cluster of differentiation 98 (CD98) and β3 integrin was determined. To further understand through which pathways the TLR 3-induced alterations occur, inhibitors were applied for Toll/interleukin-1 receptor domain-containing adaptor inducing interferon-beta (TRIF), myeloid differentiation primary response 88 (MYD88), mitogen-activated protein kinases (MAPK) and nuclear factor (NF-kB) pathways.

Results: We observed that stimulation of TLR 3 in endometrial cells with different concentrations of Poly I:C led to a reduction of the percentage of JAr spheroids attached to endometrial cells in a dose-dependent manner ($p<0.05$). This decrease was consistent in the Poly I:C treated group regardless of the co-incubation time ($p<0.05$). In addition, our results demonstrated that actin polymerization and CD98 expression significantly decreased only in response to TLR 3 activation ($p<0.05$). Activation of endometrial cells with TLR 2/6, 3 and 5 significantly reduced β3 expression ($p<0.05$). These alterations were shown to work via MYD88-MAPK pathways ($p<0.05$).

Conclusion: TLR 3 activation in the female reproductive tract influenced cytoskeletal changes and adhesion molecules expression in RL95-2 cells in vitro, which can be explained as one of the mechanisms of TLR 3-induced inhibition of trophoblast adhesion to the endometrial cells. This is a novel discovery which extends our current knowledge concerning diagnosis and treatment of viral-induced infertility cases.

Key words: *Implantation failure, Toll-Like receptors, Actin polymerization, Cluster of differentiation 98 (CD98), β3 integrin.*

Second winners *(Alphabetic order)*

A-2a

Preimplantation response to genome instability and prenatal status of genome integrity

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Introduction: Preimplantation DNA damage might alter different pathways including apoptosis; cell cycle and DNA repair. Mosaicism is prevalent in preimplantation stage. A decrease in aneuploidy rate following a prolonged co-culture of human blastocysts has been reported. Differentiation is known as the barrier for elimination of mosaicism; however some mosaicisms could be compatible with live birth.

Materials and Methods: 1) Surplus day-4 embryos of preimplantation genetic screening (PGS) candidates were classified into two groups, with and without signs

of DNA damage, to compare expression of 84 DNA damage signaling pathways genes using PCR array. 2) We used FISH to reanalyze surplus blastocysts following day 3 PGS. 3) Forty four tissues of two apparently normal fetuses were studied using microarray for mosaicism analysis following therapeutic abortion due to maternal indications. Reciprocal aberrations validated by qPCR.

Results: 1) Five of the 84 studied genes (*MSH3*, *XRCC1*, *RAD50*, *LIG1* and *CDK7*) overexpressed in embryos with signs of DNA damage. 2) Prolonged culture was not efficient to decrease aneuploidy. Mosaicism observed in 86.6% of the blastocysts; frequency of normal cells in day 7 blastocysts was lower than that of day 6. 3) Among explored Copy Number Variations (CNVs) in the tissues of the first and the second fetuses, 67 and 45 CNVs related to 13 and 14 cytogenetic locations were reciprocal, respectively. Some CNVs were limited to one or two tissues while some others were seen in several tissues.

Conclusion: 1) The altered genes are involved in DNA repair, therefore the dominant response to preimplantation DNA damage is DNA repair rather than cell cycle control or apoptosis. 2) Despite activated DNA repair pathways, the widespread abnormality in blastocysts indicates poor performance of aneuploidy correction in preimplantation stage. If apoptosis was dominant response, predictable aneuploidy was lower than what was occurred. 3) Distribution pattern of frequent CNVs indicates preimplantation origin while CNVs with low frequency likely occurred in later stages. Regarding preimplantation origin of some prenatal mosaicisms, high resolution PGS for detection of mosaic embryos in CNV level could be helpful for transfer of healthier embryos.

Key words: *Preimplantation, DNA damage, Gene expression, Prolonged culture, Mosaicism, Prenatal.*

A-2b

New insight into endometriosis pathogenesis, recurrence and treatment approach

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Introduction: Endometriosis can be regarded as a benign metastatic disease. The pathogenesis of endometriosis involves complex mechanisms such as malignant-like mechanisms. *HOX* genes are necessary for endometrial growth, differentiation and implantation and have a critical role in cancers and endometriosis. To determine cause of endometriosis recurrency, we investigated expression of 84 *HOX* genes in endometriosis compare to eutopic tissues and normal endometrium.

Materials and Methods: Samples obtained from 15 patients with endometriosis and 15 controls without endometriosis were collected. All participates were at reproductive age with normal menstrual cycles, where the same patients provided both eutopic and ectopic endometrium (endometriomas) and control samples were surgically checked for the absence of endometriosis. The expression profile of 84 genes of *HOX* family related to various aspect of cell proliferation was investigated using a qRT-PCR array. Informed consent was obtained from patients. All measurements were performed in triplicates on independent biological replicates.

Results: Expression of the 54/ 84 studied genes showed significant difference between groups. Our data showed significant over-expression of some genes which are involved in regulation of development (*SHOX*, *SHOX2*), prevention of apoptosis and promotion of cell proliferation (*DLX* 3, 4, 5 and 6), regulation of collagen expression (*MKX*) as well as *HOXC* and *HOXD* cluster in ectopic versus euotopic and control tissues, which indicated invasive property of endometriosis. Down-regulation of *HOXA* and *HOXB* cluster and some genes involved in apoptosis (*MSX1*, *MSX2*), also was observed in ectopic versus euotopic tissue.

Conclusion: Aberration in *HOX* genes expression especially genes which are involved in various aspect of cancer, including cell proliferation, invasiveness and progression, may lead to recurrent of endometriosis. So the surgeons should remove any visible implants and scar tissue with its margins, if retain any cells in the site of ectopic tissues, its can proliferate and invade. Finally it is lead to formation of a new lesion and actually recurrent of disease.

Key words: *HOX genes, Endometriosis, Recurrence.*

A-2c

Comprehensive chromosome screening of single sperm using a whole genome sequencing technique

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Introduction: About 40% of infertile men have normal semen parameters. The failure of conventional semen analysis to identify abnormalities leads to problems for the accurate characterization of infertility and, as a consequence, difficulties counseling patients and selecting optimal treatments. One factor, invisible to standard sperm assessment, which contributes to male infertility, is aneuploidy. The assessment of chromosomal aneuploidy in sperm has been challenging due to the highly condensed nature of the DNA. Fluorescent in situ hybridization (FISH) is the most common method for assessing sperm aneuploidy, but

only analyses a handful of chromosomes (typically just five). This study aimed to develop a novel protocol permitting comprehensive analysis of chromosomes in individual sperm.

Materials and Methods: 30 single sperm from a male with normal semen parameters were isolated by micromanipulation and the whole genome amplified using multiple displacement amplification (MDA). The MDA products were subjected to Next-Generation Sequencing (NGS) using an Ion Personal Genome Machine. The proportion of DNA fragments attributable to each chromosome was assessed. Excessive/deficient numbers of DNA fragments from individual chromosomes were indicative of aneuploidies. Additionally, DNA fingerprinting was applied to each MDA product, revealing any instances where two sperm had inadvertently been placed in the same sample tube.

Results: NGS permitted sequencing of the genome of each sperm to an average depth of 0.1X. All of the samples considered for NGS were confirmed to be single sperm. Analysis of the data revealed 3 chromosomally abnormal sperm. Abnormalities included +12 and +17, aneuploidies that would not be detected using standard FISH.

Conclusion: This study demonstrates the technical feasibility of NGS applied to single sperm and its advantage in providing a comprehensive chromosome assessment. The method is immediately applicable for research purposes, but is currently expensive. However, the rapidly declining costs of NGS mean that future applicability in a clinical context is likely.

Key words: *Sperm, Aneuploidy, NGS.*

Third winners*(Alphabetic order)*

A-3a

Efficacy of transvaginal perfusion of granulocyte colony stimulating factor on recurrent implantation failure: Randomized control trial

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Introduction: Repeated implantation failure (RIF) is due to poor quality of embryo, endometrium, uterine and fallopian tube. Also perinatal and immunologic factors can be noted. RIF means failure of 2 IVF cycle in patients with more than 10 high quality embryos. Granulocyte colony stimulating factor (GCSF) is a glycoprotein that stimulates cytokine growth factor and induced immune system. The aim of this study was to evaluate GCSF ability to improve pregnancy rate in women with repeated implantation failure.

Materials and Methods: This was a randomized control trial which conducted in Yazd Research and Clinical Center for Infertility, 2014-2015. Women with history of RIF and under 40 years old were included. Participants with GCSF contraindication, endometriosis and sever male factor were excluded. Totally 90 eligible women were randomly allocated in two groups. All of participants received antagonist protocol. Then 30 ml (300 mg/ml) GCSF was administered in intervention group by intrauterine infusion. Pregnancy outcomes were assessed based on chemical and clinical pregnancy.

Results: Totally 90 patients were included. The mean age of participants was 31.95±4.71 years old. There were no differences in ART and demographic characteristics of two groups ($p>0.05$). The pregnancy outcome in GCSF group was improved significantly ($p=0.043$).

Conclusion: In this RCT we could detect a significant treatment effect of GCSF on pregnancy rates. GCSF can improve pregnancy outcome in patients with RIF.

Key words: *GCSF, Pregnancy rate, Repeated implantation failure.*

A-3b

Transvaginal perfusion of granulocyte colony stimulating factor for infertile women with thin endometrium in frozen ET program: A non-randomized clinical trial

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Introduction: We often see patients with a thin endometrium in ART cycles, in spite of standard and adjuvant treatments. Improving endometrial growth in patients with a thin endometrium is very difficult. Without adequate endometrial thickness these patients, likely, would not have reached embryo transfer. We planned this study to investigate the efficacy of intrauterine granulocyte colony stimulating factor (GCSF) perfusion in improving endometrium, and possibly pregnancy rates in frozen-thawed embryo transfer cycles.

Materials and Methods: This is a non-randomized intervention clinical trial. Among 68 infertile patients with thin endometrium (-7 mm) at the 12^{th}-13^{th} cycle day, 34 patients received GCSF (300 microgram/1mL) to improve endometrial thickness by direct administration by slow intrauterine infusion using IUI catheter. If the endometrium had not reached at least to 7-mm within 48-72 hr, a second infusion was given. Endometrial thickness was assessed by serial vaginal ultrasound at the most expanded area of the endometrial stripe.

Results: The cycle was cancelled in the patients with thin endometrium (endometrial thickness below 7mm) until 19th cycle day ultimately. The cycle cancelation rate owing to thin endometrium was similar in GCSF

group (15.20%), followed by (15.20%) in the control group (p=1.00). The endometrial growth was not different within 2 groups, an improvement was shown between controlled and GCSF co-treated groups, with chemical (39.30% vs. 14.30%) and clinical pregnancy rates (32.10% vs. 12.00%) although the differences were not significant.

Conclusion: Our study fails to demonstrate that GCSF has the potential to improve endometrial thickness but it shows that GCSF has the potential to improve chemical and clinical pregnancy rate of the infertile women with thin endometrium in frozen-thawed embryo transfer cycle.

Key words: *Thin endometrium, Granulocyte colony-stimulating factor, Frozen embryo transfer, Pregnancy rate, Implantation.*

A-3c

Efficacy of motile sperm organelle morphology examination (MSOME) and sperm head vacuoles evaluation in conventional IVF versus ICSI cycles

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Introduction: The impact of MSOME criteria on ICSI outcomes is a controversial issue in the literature. There are rare studies regarding the association between anteroposterior sperm head size, cytoplasmic droplet, head shape and conventional sperm parameters. To the best of our knowledge, the prevalence of sperm deformities using MSOME has not been reported in conventional IVF cycles.

Materials and Methods: This is a prospective analysis of MSOME parameters in IVF (n=31) and ICSI cycles (n=35) performed from 2013 to 2014. MSOME parameters were evaluated as follows: vacuole: none, small, medium, large and mix; head size: normal, small and large; cytoplasmic droplet; head shape and acrosome normality. We compared the association between MSOME and conventional sperm parameters, early embryo development and pregnancy outcomes.

Results: In IVF group, the rate of large nuclear vacuole (LNV) was significantly lower in successful pregnancies compared to non-pregnant patients (7.38±4.4 vs. 13.81±9.7, respectively, p=0.045). Conversely, the rate of small nuclear vacuoles (SNVs) showed increased level in positive pregnancies. There was a positive correlation between the rates of non-vacuoles, SNVs and normal sperm shape (p<0.0001 and p=0.003, respectively). A negative correlation was found between the rate of LNVs and normal sperm shape. Moreover there was a positive correlation between progressive motility and normal head size. In ICSI group, we did not observe any association between MSOME criteria and pregnancy outcome. The rate of LNVs and large head sperm size illustrated significantly positive correlation with the percentage of non-progressive motile sperm. Also, the cytoplasmic droplet had negative effect on sperm shape (p=0.04).

Conclusion: The LNV has negative effect on sperm shape normality and pregnancy outcome in IVF cycles. However, there was no any association between MSOME parameters and early embryo development and pregnancy outcome in ICSI cycles.

Key words: *MSOME, IVF, ICSI, Sperm head vacuoles.*

A-3d

Short-term culture of human ecto-cervical epithelial cells for genomic, proteomic and functional studies

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Introduction: Understanding cell physiology is limited by reliance on tumor-derived immortalized cell lines. Primary cell culture models may offer more relevant mechanistic insight into cell physiology but are often difficult to establish and maintain. We sought to develop an optimal method for the isolation and short-term culture of human primary ecto-cervical epithelial cells (HECECs).

Materials and Methods: Fresh ecto-cervical tissues were obtained at hysterectomy and epithelia was isolated and cultured (using MEM D-Valine media to prevent fibroblast proliferation) using three different explants methods: i) tiny fragments of epithelium; ii) dissociated cells cultured after digestion using Collagenase IV and trypsin; and iii) digested tissue clumps. The epithelial phenotype of cultured cells was verified by double immunofluorescence sequential staining to detect cytokeratin, specific antigen for epithelial cells. The expression of oestrogen (ERα, ERβ) and progesterone receptors (mPRα, mPRβ, PRγ and nPRA and nPRB) genes were investigated by RT-PCR. Flow cytometry was employed to detect TLR2 and TLR4, receptor targets for our proposed functional studies of pattern recognition in the human cervix.

Results: Cultures were successfully established using all three methods but cell growth was best from digested tissue clumps which were employed for subsequent experiments. Primary cells were sub-cultured at least two times. Exclusion of fibroblasts from cultures was confirmed by the absence of staining to CD90. We confirmed the expression of all *ER* and *PR* genes, as well as the expression of TLR2, TLR4 in derived HECECs.

Conclusion: HECECs cultured from explants of digested tissue clumps, employing our protocol, yield

enough pure epithelial cell population, uncontaminated by stromal fibroblasts, which are suitable for molecular investigations involving a small number of passages.

Key words: *Cell culture, Epithelial cells, Ecto-cervix, TLR.*

A-3e

The viability rate of ovine spermatogonial stem cells after cryopreservation in different concentrations of FBS

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Introduction: Spermatogonial Stem Cells (SSCs) have the main role in spermatogenesis process. This type of stem cells possess the ability of differentiating into three germ layer lineages, so could be used for treating some kinds of infertility disorders in male patients. Also in animal reproductive technologies such as artificial insemination, transgenesis and grafting, SSCs are important supplement. For this purpose, testicular cells can be kept for long or short time. One of keeping procedures is cryopreservation, that many kinds of cryoprotectant agents are available. The higher viability rate of frozen-thawed cells, the better cryopreservation agent is.

Materials and Methods: Testicular cells were extracted from six 2-month old lambs by testicular biopsy (TESE) and bi-step enzymatic digestion. For spermatogonial stem cells and Sertoli cells quiddity confirmation, immunocytochemical analysis was used. Anti Oct-4 and anti vimentin were immunocytochemical markers. The suspended cells were collected and cultured for 12 days. The cells were frozen in two cryopreservation groups for one month in -196 degrees centigrade. The first cryopreservation group included 50% fetal bovine serum (FBS) while the second group had 70% FBS. In all groups 10% Dimethyl Sulfoxide (DMSO) was used as a cryoprotectant agent. The viability of each two types of frozen-thawed cells was assessed with trypan blue staining procedure after 1 month.

Results: There is a direct relationship between the increasing FBS concentration in cryopreservation media and the viability rate of frozen-thawed testicular cells. The viability rate of testicular cells was 64.12% for first cryopreservation group (contained 50% FBS), 67.03% for the second one (contained 70% FBS) and 89.19% for control group.

Conclusion: Based on literature, FBS containing cryopreservation media, have better cyroprotective function for SSCs cryopreserving compared to other well-known cryoprotectant agents.

Key words: *Sheep, Spermatogonial stem cell, Cryopreservation, Fetal bovine serum.*

Fourth winners *(Alphabetic order)*

A-4a

Association between nuclear receptors of estrogen and progesterone with adiponectin receptors in granulosa cells of patients with poly cystic ovary syndrome

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Introduction: The poly cystic ovary syndrome (PCOS), one of the most common endocrine disorders in reproductive age women, is associated with obesity and insulin resistance predisposing to diabetes mellitus type 2 and atherosclerosis. Adiponectin is a recently discovered adipocytokine with insulin –sensitizing and putative anti atherosclerotic properties. Several studies have illustrated that adiponectin can regulate granulosa cell stroidogenesis and the expression of genes associated with ovulation. Therefore, the aim of this study was to investigate a relationship between gene expression of estrogen and progesterone nuclear receptors and adiponectin receptors in granulosa cells (GCs) of PCOS women compared to women with normal cycling ovaries in order to achieve a better understanding of ovarian steroid status in patients with PCOS.

Materials and Methods: In this prospective study, 40 patients with PCOS and 40 women with normal ovulatory function who underwent IVF for treatment of tubal and/or male infertility were recruited. Follicular fluid was collected from patients and. GCs were isolated from follicular fluid by centrifugation and then were purified with Micro Beads conjugated to monoclonal anti-human CD45 antibodies. RNA was extracted and Reverse transcription was performed. Gene expression of AdipoR1, AdipoR2, estrogen and progesterone receptors was determined by quantitative real time PCR (q-PCR). All statistical procedures were run on SPSS 16. $P \leq 0.05$ was considered significant.

Results: By considering all subjects with and without PCOS undergoing controlled ovarian hyper-stimulation, we observed ERα and ERβ mRNA expression correlated positively with the mRNA

expression of AdipoR1 (r=0.85, p=0.0001 and r=0.92, p=0.0001, respectively) and AdipoR2 (r=0.87, p=0.0001 and r=0.88, p=0.0001, respectively). Estrogen receptor β (ERβ) expression was significantly higher compared to ERα expression in both groups (p<0.002). Moreover, progesterone receptor A (PRA) and PRB were both expressed in human GCs. However, the expression level of nuclear PRB was very low in both groups (p<0.008). There was a significant correlation between progesterone receptors and adiponectin receptors (r=0.8, p=0.0001 and r=0.88, p=0.0001). In our present results, increased ratio of PRA/PRB in women with PCOS has been revealed.

Conclusion: This research provides more evidence about expression profiles of genes involved in metabolism, steroidogenesis and ovulation in PCOS and supports the hypothesis that abnormal hormone activity, by different receptor expressions, may be an important factor in the generation of ovarian disorder.

Key words: *AdipoR1, AdipoR2, Estrogen receptor (ER), Progesterone receptor (PR), Granulosa cell (GC), polycystic ovary syndrome (PCOS).*

A-4b
GDF-9 supplementation improved embryo formation rates in clinical IVM program

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Introduction: GDF-9 is an oocyte-secreted GF which is critical for promotion of in-vitro growth of ovarian follicle and preovulatory cumulus cells (CCs) expansion. Supplementation of GDF-9 in IVM medium may enhance embryo development and fetal viability. The aim was to investigate the effects of GDF9 supplementation in IVM medium for human GV oocytes retrieved from ICSI cycles, on rates of oocyte maturation, fertilization, and subsequent embryo development.

Materials and Methods: Retrieved GV oocytes were divided in 4 groups. In group I, 108 oocytes were cultured in commercial IVM media (control); in group II, 68 oocytes were cultured with CCs; in group III, 66 oocytes were cultured in media supplemented with 200 ng/ml GDF-9; and in group IV, 99 oocytes were cultured with CCs in presence of 200 ng/ml GDF-9 at 37°C, 5% CO_2. Maturation was considered when oocytes excluded 1st polar body. Matured oocytes were screened for ZP birefringence and meiotic spindles (MS) with Polar Aide Microscopy. After ICSI, normal fertilization and cleavage rates were analyzed.

Results: Although, the maturation rate of control group (63.9%) was higher than other groups; but,in the process of fertilization (group I vs. II, p=0.01), up to embryo formation (group I vs. II, p=0.001 and group I vs. III, p=0.05) remarkable reduction was observed in group I. In term of maturation and fertilization rates, there were no significant differences between experimental groups. However, both embryo formation and quality of group III were better than the other groups. Among matured oocytes, the rates of oocytes with spindles in group III were lower than oocytes in group IV (24.6% vs. 45%, p=0.03). Whereas, the percentage of high birefringence (HB) oocytes in group I was higher than group IV (63.8% vs. 38.2%, p=0.005).

Conclusion: Application of exogenous GDF9 during clinical IVM improved embryo development. It seems a promising approach for improving human IVM program.

Key words: *Human oocyte, IVM, GDF-9, Fertilization, Embryo.*

A-4c
Effect of single dose GnRH agonist on pregnancy outcome in frozen-thawed embryo transfer cycles

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Introduction: There is no dout that luteal phase support (LPS) is essential to enhance the reproductive outcome in IVF cycles. In addition to progesterone and human chorionic gonadotropin, several studies have described GnRH agonists as LPS to improve implantation rate, pregnancy rate and live birth rate, whereas other studies showed dissimilar conclusions. All of these studies have been done in fresh IVF cycles. This prospective controlled trial was designed to test this hypothesis in frozen-thawed embryo transfer cycles (FET cycles).

Materials and Methods: In 200 FET cycles, patients were randomized on the day of embryo transfer into group 1 (n=100) to whom a single dose of GnRH agonist (0.1 mg triptorelin) was administered three days after transfer and group 2 (n=100), who did not receive agonist. Both groups received daily vaginal progesterone suppositories (800 mg daily) plus estradiol valerate 6 mg daily. The primary outcome measure was clinical pregnancy rate and the secondary outcome measures were implantation rate, chemical andongoing pregnancy rate and abortion rate.

Results: A total of 200 FET cycles were analysed. Demographic data and embryo quality were comparable between two groups. No statistically significant difference in chemical, clinical, ongoing and abortion rate was observed between the two groups (26% vs. 21%, p=0.4) and (21% vs. 17%, p=0.47).

Conclusion: Administration of a subcutaneous GnRH agonist at the time of implantation does not increase clinical or ongoing pregnancy rate in FET cycles.

Key words: *Frozen-thawed embryo transfer cycles, GnRH agonist, Luteal phase support.*

A-4d
Sperm chromatin condensation, DNA fragmentation and apoptosis in globozoospermic patients

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Introduction: Globozoospermia is a severe form of teratozoospermia with very low incidence in infertile patients that is characterized by round sperm head and lack of acrosome. It's considered as one of the important causes of male infertility which the success rate in assisted reproductive technology (ART) cycles is also low. The goal was to compare the semen parameters and chromatin/DNA integrity as well as apoptosis in ejaculated spermatozoa between globozoospermic and normozoospermic men.
Materials and Methods: In total 57 men were divided into two groups including globozoospermic (n=27) and normozoospermic men as controls (n=30). Semen analysis was performed according to WHO criteria (2010). Sperm chromatin condensation and DNA integrity were assessed using cytochemical tests including: Aniline blue (AB), Toluidine blue (TB), Chromomycin A3 (CMA3) and Sodium dodecyl sulfate (SDS) for chromatin compaction and Acridine orange (AO), Sperm chromatin dispersion (SCD) and TUNEL assays for DNA structure and apoptosis detection.
Results: There were significant differences regarding sperm count, motility and normal morphology between two groups. The percentage of abnormal chromatin packaging/ DNA integrity (using AB, TB, AO and SCD tests) was significantly higher in globozoospermic men compared to normozoospermic samples. The rate of spermatozoa with protamine deficiency (CMA3+) showed an increase in golobozoospermic patients when comparison with controls (63.59±13.29 vs. 24.17±9.5, respectively, $p<0.0001$). it should be noted that in SDS test, we didn't any significant difference between groups. But, the rate of TUNEL positive spermatozoa were significantly increased in globozoospermic cases respect to the controls (14.81±9.91 vs. 5.95± 3.02, respectively, $p<0.0001$). There was no significant correlation between sperm DNA denaturation, DNA fragmentation and apoptosis in globozoospermic men. Our data showed significant correlation between single strand DNA (AO+) and progressive motility ($p=0.036$).
Conclusion: The rate of spermatozoa with abnormal chromatin packaging, DNA damage and apoptosis were significantly higher in globozoospermic samples than normal fertile men. However, the sperm chromatin/ DNA anomalies may be considered as one of the main etiologies of ART failure in these patients.

Key words: *Globozoospermia, Male infertility, Sperm chromatin, DNA integrity.*

A-4e
Does cosmetic micromanipulation of vitrified-warmed preimplantation embryos enhance pregnancy rate?

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Introduction: Cytoplasmic fragmentation in cleaving embryos which is cornerstone of each embryo grading system has been shown to be an important biomarker for implantation potential. Beside fragmentation, another dysmorphism is the presence of coarse granulation in perivitelline space (PVS). In our hypothesis, the cosmetic micromanipulation (CM) is defined as removal of fragments, coarse granules, and attached cumulus cells (CCs) to zona pellucida from the vitrified- warmed embryos before embryo transfer (ET). The objective was to investigate the effect of CM on the subsequent cell division, morphology and pregnancy outcomes of the vitrified-warmed fragmented human embryos.
Materials and Methods: Patients undergoing frozen ET (FET) with similar clinical characteristics were included in this ongoing prospective randomized study. They were divided into three groups of CM, laser assisted zona hatching (LAH) and control. The vitrified-warmed embryos with >10% and <50% fragmentation met inclusion criteria. In CM group, five hours after embryo warming and morphology evaluation, the embryos were subjected to CM after LAH .Whereas; in LAH group warmed embryos were subjected to LAH only. After overnight incubation, cell division and morphology of embryos were evaluated and divided embryos were transferred.
Results: The morphological grade of fragmented embryos improved after the CM. Most of the fragmented embryos did not show a regeneration of fragments after CM during the subsequent development, and a beneficial effect of CM on the development of the embryos was observed. Pregnancy rates in CM, LAH and control groups were 42.8%, 37.5% and 45.4% respectively. There were no statistical significance ($p>0.05$) in pregnancy rates between the groups because of the low number of trials.
Conclusion: CM improved the subsequent development as well as the morphological grades of fragmented embryos. But, our preliminary data showed that this technique in FET cycles neither compromise nor improved pregnancy outcomes in unselected patients. Further controlled trials will determine whether CM can improve pregnancy outcomes in a selected patient population, such as recurrent implantation failure and advanced maternal age.

Key words: *Cosmetic microsurgery, Fragment removal, Pregnancy, Vitrified-warmed embryo.*

Oral Presentations

O-1

Factors associated with adoption acceptance rate from the view point of infertile couples

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Introduction: Nowadays artificially assisted reproductive techniques are used to cure infertility. These methods are highly expensive, time-consuming and have low success rates which are usually around 20-40%. One of the best alternate methods for infertility treatment that can be considered is adoption that often decreases the treatment costs and the psychological impact within an infertile couple.

Materials and Methods: A cross-sectional study was performed between October 2009-2010 on 200 infertile couples who had been referred to Infertility Center of Shahid Sadoughi University of Medical Sciences. Information gathered through face-to-face interview and questionnaires. The data analyzed through a SPSS software program using ANOVA test.

Results: There was a significant statistical relationship between adoption acceptance value scores and marriage duration of a couple (p=0.002 in men, p=0.004 in women) and presence of adoption backgrounds in male relatives (p=0.004). There was no statistically significant relationship between age, gender, education level, and onus of infertility, the number of previous referrals for an infertility solution and presence of adoption backgrounds in female relatives.

Conclusion: Adoption as an alternative option to infertility treatment need to be more considered as a medical, social and cultural issue.

Key words: *Infertility, Adoption, Artificially assisted reproductive techniques.*

O-2

Macrophage migration inhibitory factor as a potential biomarker of endometriosis

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Introduction: In endometriosis, there are an increased number of activated macrophages in the peritoneal fluid. The macrophages existing in the inflammatory areas secrete Macrophage Migration Inhibitory Factor (MIF). MIF via its receptor, CD74, initiates a signaling cascade that leads to proliferation and survival of cells. MIF binding to CD74 activates p38 signaling pathways that lead to positive effect on the expression of COX-2. The aim of this study was to evaluate the expression of MIF, CD74, and COX-2 in normal, ectopic, and eutopic endometrium during the menstrual cycle and to assess MIF level in peripheral blood.

Materials and Methods: All women taking part in this study were between 20-45 years old, had no endometrial hyperplasia or neoplastic. In total 20 ectopic and 20 eutopic endometriosis tissues and 12 normal endometriums during menstrual cycle as control group were tested in this study. Peripheral blood samples were likely obtained from each group. The expressions of MIF, CD74, and COX-2 in normal, ectopic, and eutopic endometrium were evaluated with the use of real-time polymerase chain reaction. MIF protein in peripheral blood samples was checked with the use of ELISA.

Results: Relative mRNA expression of MIF, CD74, and COX-2 were significantly higher in ectopic endometrium than in eutopic and control endometrium. Also, there were significant differences in expression of these genes in normal, ectopic, and eutopic endometrium during the menstrual cycle. Moreover, women with endometriosis had significantly higher circulating levels of MIF compared with control subjects.

Conclusion: Dynamic expression of MIF, CD74, and COX-2 during the menstrual cycle could play an essential role in reproduction, inflammation, and endometrium reconstruction. A higher expression of these genes in ectopic endometrium can be considered as a molecular biomarker for endometriosis development and pathophysiology. Also, high level of MIF in blood serum can act as a biomarker in the diagnosis of endometriosis.

Key words: *MIF, CD74, COX-2, Endometriosis.*

O-3

Expression and epigenetic alterations of aromatase coding gene, *CYP19A1*, in cumulus cells of infertile endometriosis patients

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Introduction: Endometriosis, an estrogen-dependent disease, has adverse effects on all aspects of reproductive process. Aromatase, the key enzyme of estrogen biosynthesis, is encoded by the *CYP19A1* gene. Aromatase plays a pivotal role in ovarian functions, folliculogenesis and acquisition of oocyte competence. Among the various promoters of *CYP19A1*, the promoter PII is the most active ones in ovarian cells. Previous studies showed that changes in gene expression of aromatase are associated with pathogenesis of endometriosis but no epigenetic marks have been reported for aromatase regulation in cumulus cells (CCs) of endometriosis till date. The purpose of this study was to answer the following questions: the first, does endometriosis alters *CYP19A1* gene expression in CCs of endometriosis patients? The second, is there any association between altered *CYP19A1* gene expression and epigenetic alterations of its promoter region?
Materials and Methods: Case-control study was conducted on 10 infertile endometriosis patients and 10 patients with tubal factors of infertility who underwent ovarian stimulation with GnRH agonist for intracytoplasmic spermatozoa injection (ICSI). Cumulus oocyte complexes (CC) were obtained from follicles during ovarian puncture. Only the CCs from MII oocytes were selected for this study. Total RNA extraction and cDNA synthesis were performed using Micro-RNeasy and QuantiTect Whole-Transcriptome Kits, respectively. Relative expression of *CYP19A1* gene was examined by Quantitative real-time PCR. The DNA binding of MeCP2 and specific histone modifications in PII promoter region of *CYP19A1* gene were examined by Chromatin Immunoprecipitation (ChIP) assay.
Results: Our data revealed that the mean relative expression of *CYP19A1* gene was significantly lower in CCs from infertile endometriosis patients compared with the control group ($p<0.05$). In CCs of endometriosis patients, incorporation of MeCP2 on promoter PII of CYP19A1 is significantly higher than that of control group ($p<0.05$). Furthermore, a significant hypoacetylation at lysine 9 of histone 3 (H3K9ac) of promoter PII was observed in patients affected endometriosis, whereas no significant difference of methylation level at lysine 9 of histone 3 (H3K9me2) was detected between patients and control groups.
Conclusion: For the first time our results have shown that decreased CYP19A1 expression in cumulus cells of endometriosis patients might be the result of epigenetic alterations in regulatory region of *CYP19A1*, either through DNA methylation or histone modifications. Changes in gene expression of aromatase may impair the development of the follicles and follicular steroidogenesis leading to poor oocyte quality and maturity in endometriosis patients. These alterations may have close relationship with endometriosis-associated infertility.
Key words: *Endometriosis, Aromatase, Epigenetic, Cumulus cell.*

O-4
Evaluation of immunological interaction between spermatozoa and fallopian tube epithelial cells

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Introduction: Toll-like receptors (TLR) are one of the major compartments of innate immune system. It was revealed that the TLR have relevance in ovulation, sperm capacitation and fertilization. So, in this study, the expression of TLR, their adaptor molecules and cytokines in human fallopian tube cell line under the effect of human normal spermatozoa was evaluated.
Materials and Methods: TLR mRNA and protein were evaluated in OE-E6/E7 cell line. Semen samples from 10 donors were collected and co-incubated with OE-E6/E7 cell line and used as sperm group, and cell line without spermatozoa was used as control group. Afterwards, the level of TLR, their adaptor molecule and cytokine mRNA expression was compared using qPCR in sperm and control groups, and supernatant was used for ELISA assay of IL-6, IL-8, TNF-α and IFN-α. To determine whether elevated cytokine reaction to spermatozoa in OE-E6/E7 cell line is mediated via TLR, TLR3 function-blocking antibody was used.
Results: OE-E6/E7 cell line expressed TLR1-6 genes and proteins. TLR expressions, especially TLR3 and TLR5, in OE-E6/E7 cell line under the effect of spermatozoa were significantly higher. Also, levels of adaptor molecules and cytokine production were increased in sperm group than in control group ($p<0.05$). Using TLR3 function-blocking antibody confirm that cytokines production were due to TLR3 stimulation by sperm.
Conclusion: It may be hypothesised that TLR are essential for spermatozoa and fallopian tube immunological interaction. IL-6, IL-8 and IFN-β have many physiological roles in fallopian tube, in addition to protecting it against invading pathogen, which is really important in reproductive system especially in fallopian tube that is susceptible to infections.
Key words: *Fallopian tube, Innate immunity, Spermatozoa, Toll-like receptor.*

O-5

Evaluation of In vitro growth and apoptosis incidence in vitrified human ovarian tissue following treatment with growth differentiating factor 9B (GDF-9B) and Leukemia inhibitory factor (LIF)

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Introduction: The conventional freezing and vitrification are different cryopreservation protocols for fertility preservation in cancer patients. The high effectiveness of vitrification for human oocytes and embryos is shown, whereas data on human ovarian tissue are limited. The objective was the assessment of follicular growth, ultrastructure, and apoptosis incidence in human ovarian tissue following vitrification/warming and after culture in the presence of GDF-9B and or LIF.

Materials and Methods: Biopsies of ovarian cortex from normal pregnant women divided to 2 main groups: vitrified and non-vitrified and some of fragments in both groups culture in presence and absence of GDF-9B or LIF. Then the morphology, ultrastructure and incidence of apoptosis using TUNEL and DNA Laddering and caspase 3/7 assay and analysis of apoptosis related genes expression in ovarian tissue fragments were evaluated before and after 2 weeks culture.

Results: Morphology and ultrastructure of vitrified human ovarian tissue were similar to vitrified group and were well preserved. Apoptosis evaluation assessments (DNA Laddering, TUNEL, Caspase-3/7 activity, apoptotic genes expression) in both non-vitrified and vitrified groups showed no significant differences. Morphological studies of ovarian tissue in LIF or GDF-9B treated groups showed better conservation of ovarian follicles ($p<0.05$). But there were no significant differences between non-vitrified and vitrified ovarian tissue in both LIF and GDF-9B treated groups. The levels of 17-β estradiol and progesterone were higher and DHEA was lower than other cultured groups. Apoptosis evaluation techniques showed that apoptosis incidence in GDF-9B or LIF treated groups were lower than non-treated cultured groups and non-cultured ovarian tissue ($p<0.05$).

Conclusion: We concluded that vitrification of human ovarian tissue has not increased the incidence of apoptosis and LIF as an antiapoptotic factor could improve survival and development of cultured follicles and reduce incidence of apoptosis in ovarian tissue.

Key words: *Vitrification, Human ovarian tissue culture, GDF 9B, LIF, Apoptosis related genes.*

O-6

Testis development in the absence of SRY: chromosomal rearrangements at *SOX9* and *SOX3*

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Introduction: 46,XX disorders of sex development (DSDs) are congenital conditions in which, in the presence of a female karyotype, the development of gonadal and anatomical sex is atypical, ranging from various degrees of ambiguous genitalia to phenotypic males with azoospermia.

Materials and Methods: We analyzed, by conventional and molecular cytogenetics, 19 novel SRY-negative unrelated 46,XX subjects both familial and sporadic, with isolated DSD. Collectively in our cohort of 19 novel cases of SRY-negative 46,XX DSD.

Results: One of the cases had a de novo reciprocal t(11;17) translocation. Two cases carried partially overlapping 17q24.3 duplications ~500 kb upstream of *SOX9*, both inherited from their normal fathers. Breakpoints cloning showed that both duplications were in tandem, whereas the 17q in the reciprocal translocation was broken at ~800 kb upstream of *SOX9*, which is not only close to a previously described 46,XX DSD translocation, but also to translocations without any effects on the gonadal development. A further XX male, ascertained because of intellectual disability, carried a de novo cryptic duplication at Xq27.1, involving SOX3. CNVs involving *SOX3* or its flanking regions have been reported in four XX DSD subjects.

Conclusion: We report additional evidences suggesting that, in the absence of SRY, altered expression of genes crucial to gonadal development, such as *SOX9* and *SOX3*, may invert the expected embryonic plan. Whereas for *SOX3*, it is easier to envisage a direct link between its duplication and increased gene expression,16 it is more difficult to understand the true functional link between duplications upstream of *SOX9* and the different abnormal phenotypes, including gonadal abnormal differentiation. Our study reports that the incidence for RevSex copy number gains associated with SRY-negative isolated 46,XX DSDs is 410%. We can speculate that the RevSex duplication causes increased expression of SOX9 in undifferentiated gonadal cells, thus, resulting in testis differentiation even in the absence of SRY. In fact, duplications of SOX9 are associated with XX sex reversal not only in transgenic mice 9 but also in the recently reported case of a deer, 38 and in three cases of dogs. 39 Our case 3 shows that also interruption of the region upstream to the RevSex can result in XX sex reversal. Altogether our data reinforce the role of the desert region upstream of *SOX9* in the regulation of this gene, as indicated by an altered histone methylation signature demonstrated in

one of the RevSex duplicated cases. It is noteworthy that RevSex includes two lncRNAs, TCONS_00025195 and TCONS_00025196, with specific expression in the testis, possibly having a role in *SOX9* transcriptional regulation.

Key words: *SRY, Testis development, SOX9, SOX3.*

O-7

Effect of 655 nm diode LASER irradiation on human sperm cell motility and ROS (Reactive Oxygen Species) production

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Introduction: Sperm motility is known as an effective parameter in male fertility and it depends on energy consumption. Low-level LASER irradiation could increase energy supply to the cell by producing of adenosine triphosphate (ATP).

Materials and Methods: Sperm motilities are assessed by means of Computer-Aided Sperm Analysis (CASA), and ROS levels are evaluated by chemiluminescence (CL) technique; all according to the WHO 2010 manual. Data analysis was performed using SPSS software and GEE analysis, and statistical significance was set at $p<0.05$. In total 25 human semen samples of asthenospermic patients (25-45 years old) with appropriate volume (4 ml) were used in this study. The patients were referred to the Royan Infertility Center for the first time. They were seeking for infertility treatment and had received no medication before. All samples were collected in special containers and treated for routine Semen Analysis according to the WHO 2010 manual. Fresh human semen specimens were divided into 4 equal portions, irradiated by 655 nm diode GaInAlP LASER irradiation with varying doses as: 0 (control), 4, 6 and10 J/Cm^2. At the time of 0, 30, 45 and 60 min following irradiation, sperm motilities and ROS levels were assessed in all samples.

Results: LASER irradiation could increase sperm motility but it did not have any significant effect on ROS production in sperms. Sperm motility of the control groups significantly decreased after 30, 45 and 60 min of irradiation time, while in the irradiated groups remained constant or slightly increased. Significant increases have been observed in dose of 10 J/cm^2 at the time of 60 min. ROS levels in irradiated groups slightly increased in comparison to control groups, but it was not statistically significant.

Conclusion: These results suggest that irradiating human sperms with 655 nm diode laser at 4, 6 and 10 J/cm^2 energy density doses can improve their progressive motility which may be related to increasing of energetic efficiency. The maximum effect appears on dose of 10 J/cm^2, and at the time of 60 min after irradiation. The results of ROS levels assessment in control and irradiated groups showed that LASER irradiation did not have harmful effects like oxidative stress on sperm cells.

Key words: *Sperm cell motility, Laser irradiation, Reactive Oxygen Species (ROS).*

O-8

Effect of Melatonin on cryopreservation-induced oxidative stress and apoptosis in human spermatozoa

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Introduction: Sperm cryopreservation is an important part of fertility preservation and Assisted Reproductive Techniques (ART). However cryopreservation due to increase of Reactive Oxygen Species (ROS) generation and apoptosis, can exerts undesirable effects on sperm motility, sperm viability, sperm morphology, and eventually, influence on fertilization and pregnancy rate. Many studies reported that melatonin has antioxidant and scavenging activities, but its antioxidant effects on sperm are rather contradictory. The aim of the present study was to investigate the protective effect of melatonin on sperm function during cryopreservation.

Materials and Methods: Liquefied semen samples were collected from normozoospermic men (n=21) who were undergoing semen analysis for couple infertility in the Andrology Laboratory of Dr. Shariati Hospital, Tehran, Iran. After preparation by double wash (400× gr, 5 min) swim-up technique, the samples were divided into two aliquots: 1) Freeze without treatment as control 2) melatonin treated. Both groups were stored in liquid nitrogen for two weeks, and then were thawed. Motility was evaluated by means of CASA. Reactive oxygen species (ROS) and apoptosis were assessed by flowcytometry and ELISA (Caspase 3 activity assay kit) respectively.

Results: Our results indicate that melatonin appreciably increased mean total motility (45.75±5.75 vs. 38.37±3.25 $p<0.04$). Moreover, the percentage of both DCFH-DA (H_2O_2) and DHE (O_2^-) positive cells was decreased significantly (76.00±2.27 vs. 67.06±2.23, $p<0.03$) and (41.39±2.40 vs. 31.27±1.74 $p<0.02$ respectively) in comparison to the control group. Caspase3 activity were also significantly higher in control group compared to melatonin treated group (2.13.00±0.24 vs. 1.40±0.17, $p<0.04$).

Conclusion: These results suggest that the addition of melatonin to cryopreservation medium increase post-

thaw sperm quality and decrease sperm apoptosis which may relate to a reduction in sperm ROS level.
Key words: *Melatonin, Cryopreservation, ROS, Caspase.*

O-9
Effect of human ovarian tissue vitrification on the expression of developmental genes

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Introduction: Ovarian tissue cryopreservation is an alternative strategy to preserve the fertility of women predicted to undergo premature ovarian failure due to cancer treatment, genetic disorders or other certain diseases. This approach has the advantage of restoring of both fertility and endocrine function, and may be the only acceptable method to preserve fertility for pre-pubertal girls.
Materials and Methods: Human ovarian tissue samples were collected from five transsexual patients. In the laboratory, medullary part was removed by surgical blade and the cortical tissue was cut into small pieces. Some pieces were vitrified and warmed and the others were considered as non-vitrified group (control). Follicular normality was assessed with morphological observation by a light microscope, and the expression of *Figla*, Kit ligand, *Gdf9*, and *FSHR* genes was examined using real-time q-PCR in both the vitrified and non-vitrified groups.
Results: A total of 510 follicles were counted and analyzed in both the vitrified and non-vitrified tissues (200 follicles in the vitrified and 310 follicles in the non-vitrified tissues). Overall, 85% of the follicles preserved normal morphologic feature and 15% of them were degenerated after warming. Among normal follicles, the proportion of primordial, primary and secondary follicles was 57.7%, 25.2% and 2.1%, respectively. The percentage of normal follicles and the expression of *Figla*, Kit ligand, *Gdf9*, and *FSHR* genes were similar in the vitrified and non-vitrified groups ($p>0.05$).
Conclusion: Our results for the first time demonstrated that in spite of some alterations in morphology of human ovarian tissue after vitrification using DMSO, EG and sucrose no remarkable effect on the expression of developmental genes was observed immediately after warming.
Key words: *Vitrification, Folliculogenesis, Gene expression, Human, Ovarian cortex.*

O-10
Fertility preservation of young women with endometrial carcinoma or complex atypical hyperplasia: Case series and literature review

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Introduction: Although endometrial cancer is primarily a postmenopausal disease, 25% of patients are in premenopausal age with 3-5% being 40 years old or younger who have infertility or desire to preserve their fertility. The younger groups of women with endometrial carcinoma are frequently null gravid with a history of infertility and strong desire to preserve fertility, which may pose a therapeutic dilemma for both patients and physicians.
Materials and Methods: The study has been done within 2008-2014 in Gynecological Oncology Department and Research and Clinical Center for Infertility of Shahid Sadoughi University of Medical Science, Yazd, Iran. All of young women who were in reproductive age (15-45 years) and desired to preserve their fertility entered to the study. All of patients were diagnosed endometrial carcinoma or complex atypical hyperplasia. All of patients underwent pelvic MRI with and without contrast for evaluation of uterine involvement. If they had early stage endometrial carcinoma without myometrial invasion, we suggested hormonal therapy (megestrol 40-160 mg oral or Diphereline 3.75 mg IM every 28 days for 3 months) after getting informs consent. All of them underwent dilatation and curettage after 3 months hormone therapy. We evaluated 12 young women with atypical complex hyperplasia or early-stage endometrial cancer that were treated with conservative hormone therapy.
Results: The mean of age was 29.7 years (15-45). Two patients were virgin. Five patients had endometrial adenocarcinoma and seven had complex atypical endometrial hyperplasia. All of patients treated by megestrol (2-3 tablet in day) for 3 months firstly. One patient did not answer to one period of Megestrol and we followed treatment by 3 months Megestrol high dose (160 mg) and then Diphereline 3.75 IM for 3 months. These patients had normal pathology after 3 periods of 3 months treatment. All of patients had normal menstruation one of them who needed 4 times curettage. Unfortunately she had atrophic endometrial and for childbearing she was suggested to get uterine surrogacy. But the other patients did not have any problem in menstruation and one of them except had one baby after fertility preservation.
Conclusion: Hormone therapy has been proposed for young women with endometrial cancer (grade 1) who wish to preserve their fertility. However, detailed

evaluation including physical examination, history taking, performing D & C, examining the specimen by a skilled pathologist, using imaging techniques, especially contrast enhanced MRI and for some patients explorative laparoscopy with sampling of peritoneal and lymph nodes, and evaluation of adnexa is necessary. Also for patients in stage I/ grade 1, advisory sessions on the benefits and side-effects of high-dose progesterone with evaluation of the endometrium every three months until total regression is recommended. After childbearing we suggest TAH+BSO for prevention of endometrial, ovarian and breast cancer.

Key words: *Endometrial cancer, Complex atypical hyperplasia, Young women, Fertility preservation.*

O-11
Cosmetic micromanipulation of preimplantation embryos enhances pregnancy rate in patients with previous implantation failure

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Introduction: In ART clinics, the best embryos are selected according to the morphology criteria on embryo transfer (ET) day. Beside cytoplasmic fragmentation which is cornerstone of each embryo grading system, another dymorphism is the presence of coarse granulation around the blastomeres and attached cumulus cells (CCs) to zona pellucida. So in our hypothesis, the cosmetic microsurgery is defined as removal of fragments, coarse granulations, and CCs from the embryos pre ET. We sought to evaluate the effect of cytoplasmic fragment removal and coarse granulation removal from PVS and detachment of CCs (cosmetic micromanipulation) from the embryos before ET on pregnancy outcomes in patients with and without implantation failure (IF).
Materials and Methods: 90 ICSI cycles with male factor infertility were included in this ongoing prospective randomized study that were aliquot into three groups of case (n=30), control (n=30), and sham (n=30). Each group was further divided into two sub-groups of with and without IF. The embryos with >10% and <50% fragmentation met inclusion criteria. In case group, the embryos were subjected to fragment removal, coarse granulation removal or detachment of CCs before ET. In sham group, the embryos were subjected to laser assisted zona hatching only. The removed fragments were analyzed by TEM for ultrastructural assessment. The detached CCs to embryos on ET day and denuded CCs on day of ICSI were considered as case and control groups, respectively. The expression of Bcl2, Bax, Caspase 3 and GAPDH were analyzed by real time RT-PCR in CCs of control and case groups after total RNA extraction and cDNA synthesis.
Results: There were no significant differences for patients' age, duration of infertility, levels of serum estradiol, LH, FSH, type of ovarian stimulation, number of cumulus oocyte complexes, MII oocytes, fertilized oocytes, formed embryos and transferred embryos between the groups. The pattern of fragments (localized and distributed), blastomere evenness, and percent of fragmentations were similar between groups. The pregnancy rates showed no significant differences between the groups in patients without IF. 70% pregnancy rate was achieved from case compared to 10% pregnancy rate in controls (p=0.02) and 33% in sham group in cycles with previous IF. Preliminary micrographs from TEM showed the presence of vacuoles and cortical granules in fragments. The rate Bax, Bcl2, Bax/Bcl2 and Caspase 3 showed an increasing trend in case group compared to controls, but the difference was not significant.
Conclusion: The preliminary data generated from this study showed that human embryo cosmetic micromanipulation can improve the pregnancy outcomes in patients with previous IF. Currently, this technique is not recommended for all ICSI cases.

Key words: *Cosmetic microsurgery, Fragment removal, Pregnancy.*

O-12
Morphology and apoptosis evaluation in long - term cultures of vitrified mouse whole ovaries in the percent of LIF

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Introduction: The ovary is composed of several stages of follicles having shown different tolerance to cryodamage. Some reports demonstrated that small follicles were well preserved during vitrification and warming process, however in vitro culture and the development of these small size follicles is problematic and many attempts have focused their attention on improving their in vitro growth. LIF is a glycoprotein that presents in follicular fluid and supports the initiation of in vivo or in vitro follicular growth.
Materials and Methods: The vitrified and non-vitrified ovaries of one-week old mouse were cultured in the presence or absence of LIF for 7 days. The development of ovarian follicles was studied by hematoxylin-eosin staining. The mean area was analyzed and apoptosis assessment was done using the transmission electron microscopy, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) method, DNA laddering and caspase -3/7 activity technique at the beginning and at the end of culture period in all groups of study. The hormonal assay was done on the collected media during culture period.
Results: The proportion of preantral follicles and the levels of hormones were increased in all cultured groups and it was significantly higher in LIF treated groups

than their control (p<0.001). The ultrastructural characteristics of cell death, DNA fragmentation and TUNEL positive signals were prominent in vitrified cultured ovaries. The level of caspase -3/7 activity was higher in vitrified cultured ovaries.

Conclusion: LIF treatment appeared to significantly increase the follicular development during 7 days of culture of both vitrified and non-vitrified ovaries. The highest proportions of apoptotic follicles and stromal cells were observed in the vitrified ovaries after culture.

Key words: *Apoptosis, In vitro culture, Leukemia inhibitory factor, Vitrification.*

O-13

Relationship between equilibration times and the presence of cumulus cells for vitrification of in vitro matured ovine oocytes

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Introduction: Exposure time to the cryoprotectant solution before cooling is an important factor for successful vitrification and it has been suggested that the presence of cumulus cells surrounding the oocyte is beneficial for subsequent development of matured oocytes after vitrification. We evaluate best equilibration time for vitrification of oocytes with cumulus cells (MIICOCs) and without cumulus cells (MIIDOs).

Materials and Methods: In this study, COCs with a compact cumulus investment was used and then GV oocytes were matured. MIICOCs or MIIDOs were subjected randomly to the equilibration solution for 5, 7, or 10 min prior to vitrification. The effect of equilibration time on post-vitrification development (Viability, cleavage and blastocyst rate) of embryo was assessed.

Results: In the current study there was no difference in survival rates of vitrified-warmed oocytes equilibrated at different times. Although in MIICOCs group there was a trend of an increased cleavage rate as the equilibration time was increased. Moreover, the highest cleavage rate in MIICOCs (55%) and MIIDOs (55%) groups were achieved after 10 and 7 min equilibration, respectively.

Conclusion: It seems that the oocytes enclosed with cumulus cells have needed more equilibration time compared with the cumulus-free oocytes. The results show that the optimal exposure time to achieve survival after vitrification depends on the presence or absence of cumulus cells.

Key words: *Ovine, Mature oocytes, Vitrification, Equilibration time, Cumulus cells.*

O-14

Most important challenges and interventional strategies in providing reproductive health services for prevention, diagnosis and treatment of sexually transmitted diseases in view of reproductive health care providers in health care centers of Yazd, Iran in 2014

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Introduction: The World Health Organization reports the prevalence of Sexually Transmitted Diseases in the world has been raised. Sexually Transmitted Diseases are the major cause of illness among young men (15-24 yr) and the second most important cause of maternal morbidity among young women in developing countries. Due to the importance of the disease and its complications such as fetal death, neonatal period and infancy infections, infertility, ectopic pregnancy, anogenital cancer and finally death, lack of prevention and control, early diagnosis and treatment result in serious health consequences. Health care centers are the first place for referring most of women and men for getting reproductive health care services including infertility, in Iran.

Materials and Methods: In a sequential exploratory mixed method Delphi study (qualitative and quantitative study), which was conducted in two phases between March 2013 and December 2014, in total, 35 academics, clinicians, faculty members and reproductive health providers in various related disciplines to reproductive health care including; epidemiology, reproductive health, medicine, midwifery and public health were purposively selected as expert panel members. In the first phase of the study (qualitative study) data were gathered through completing a questionnaire including open-ended questions regarding challenges and interventional strategies in providing reproductive health services for prevention, diagnosis and treatment of Sexually Transmitted Diseases by expert panel members and responses were analyzed using Qualitative Conventional Content Analysis. In the round 2 Delphi, the draft of questionnaire regarding challenges and interventional strategies developed in round 1, delivered again to the expert panel members who had participated in the first round. Finally in round 3 Delphi (quantitative study), percentage of expert panel members agreement (Consensus percentage) towards challenges and interventional strategies in providing reproductive health services for prevention, diagnosis and treatment of Sexually Transmitted Diseases were determined using descriptive statistical tests.

Results: Mean age of expert panel members was 38.8±8.88 years old. The mean length of their work experience was determined 15.22±7.87 years. 97.1% of

the expert's panel members were female. The most important challenges for provision of reproductive health services in Sexually Transmitted Diseases in view of experts panel members were respectively: Increasing high risk sexual behaviors with 94% agreement of expert panel members, lack of treatment due to economic problems 90%, clients concerns for un respecting their rights to confidentiality 88%, clients, late referring to health care centers 86%, clients unawareness regarding nature of disease transmission via sexual relationship 83% and clients, rejection due to condom consumption 81%. Interventional strategies in view of experts panel members for aforementioned challenges were determined in sequence: Provision of necessary educations to the client's 88%, emphasis on protection activities in order to partner safety 88%, emphasis on treatment for avoiding serious health consequences 88%, emphasis on treatment in both sexes 87%, providing appropriate professional relationship with clients 86%, provision information in premarital consultation program 85% and reform in health insurance system 76%.

Conclusion: The results of study indicated multiple challenges in on time and appropriate prevention, diagnosis and treatment of Sexually Transmitted Diseases. So, effective and integrated interventions and activities are required including spousal education, instruction via mass media programs, effective and comprehensive consultation agenda and reform in insurance payment system.

Key words: *Sexually transmitted diseases, Challenges, Interventional strategies, Reproductive health, Health care centers.*

O-15
Isolation of Spermatogonial Stem Cells from Tumoral cells by drug delivery

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Introduction: Testicular cancer is the most common cancer affecting men of reproductive age. Cisplatin is one of the majority helpful chemotherapeutic agents for treatment of this cancer. In addition, spermatogonial stem cells (SSCs) are necessary for the improvement of spermatogenesis subsequent of exposure to cytotoxic agents such as cisplatin. The aim of this study was to evaluate the anticancer activity of cisplatin-loaded PLGA nanoparticles on mouse malignant testicular germ cell line (EL-4) and spermatogonial stem cells in vitro.

Materials and Methods: The isolated spermatogonial cells were co-cultured with EL-4 cells. Then, cells were divided into six culture groups: Control (culture in DMEM/ F12 containing 1% FCS, 10 ng/mL GDNF and 20 ng/ml bFGF), Sham (basic media with 0.1% DMSO) and Experimental groups. Co-cultured cells in experimental groups were treated with different doses of cisplatin (5 µg/ml, 10 µg/ml, 15 µg/ml) and cisplatin-loaded PLGA nanoparticles by effective dose for 12, 24, 48 and 72 hr and then, the cells culturing in media continued for 2 weeks with basic media. The nanoparticles were prepared by W/O/W double emulsion-solvent evaporation technique. After characterized, they were targeted with foliate. In vitro release characteristics, stability, drug loading and loading efficiency were studied. DLS data showed that the mean diameter of PLGA nanoparticles were ranging between 100-150 nm. The particles were investigated by SEM and TEM to observe surface topography and the morphology. Percentage of Cells was assayed after treatment using Flow cytometry assay.

Results: This result was associated with a higher activation of apoptosis in EL-4 cells especially in experimental groups were treated with cisplatin-loaded PLGA nanoparticles.

Conclusion: The PLGA nanoparticles seem to provide a promising carrier for cisplatin administration, which was consistent with a higher activation of apoptosis than free drug.

Key words: *Cisplatin, PLGA nanoparticles, Spermatogonial stem cells.*

O-16
3D Sonohysterography for the investigation of female infertility: Technique and application

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Introduction: 3D Sonohysterography (3D-SHG) is a recent imaging technique for assessment of uterine cavity and myometrium to detect causes of female infertility.

Materials and Methods: A review was performed within articles published at "PubMed", "Elsevier", "Google Scholar", "EBSCO", original text books etc. to reach the aim. Many unique high-quality 2D/3D hysterosonograms are provided in this article, using the archive of infertile patients who underwent SHG at imaging department of Royan institute, Tehran, Iran.

Results: Sonohysterography involves the slow infusion of sterile saline solution into the uterus during ultrasound imaging. Expansion of endometrial cavity on SHG allows optimal visualization of the endometrium and plays an important role in the investigation of abnormalities related to the uterine cavity. Uterine abnormalities that can be detected by SHG were grouped into congenital uterine anomalies (arcuate, septate, subseptate, unicornuate, bicornuate and didelphys uteri) and acquired endometrial abnormalities (polyps, hyperplasia, leiomyomas, and intrauterine adhesions). SHG is shown to be accurate and reliable in the investigation of these pathologies via several studies. Thus, proper application of which can reduce indications for diagnostic hysteroscopy during infertility workup. In this article, we provided lots of unique hysterosonograms to describe about the instruction of SHG for obstetricians and radiologists working at the infertility treatment centers.
Conclusion: 3D Sonohysterography is an accurate, non-invasive, and cost-effective tool that helps obstetricians to evaluate uterine causes of female infertility, to save the time and make better treatment choices.
Key words: *Female infertility, 3D Sonohysterography, Uterus, Endometrium.*

O-17
First successful pregnancies following embryo selection using Time Lapse technology in Iran: Case reports

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Introduction: Embryo selection is a vital part of in vitro fertilization (IVF) programs, with morphology-based grading systems having been widely used for decades. Time-lapse imaging combined with embryo morphokinetics may proffer a non-invasive means for improving embryo selection. We report the first ongoing and chemical pregnancies using Time lapse embryo scope to select best embryos for transfer in Iran.
Case: A case with tubal factor infertility was admitted to IVF program with normozoospermia. After ovarian hyperstimulation, 7 COCs were retrieved and inseminated with 25,000 progressive sperms/ oocyte. 6 zygotes were placed individually into the micro wells of equilibrated embryo scope dish for time-lapse observation, and incubated at 37°C, 5% CO_2. On day 3, single embryo transfer (SET) took place based on kinetic parameters of the embryos. Clinical pregnancy was confirmed 7 weeks after SET. The second case with history of previous ICSI failure was admitted with azoospermia. 9 MII oocytes underwent ICSI, and incubated in Time lapse facilities. The rest of procedures were followed as described for case 1. Chemical pregnancy was confirmed 15 days after SET.
Conclusion: This approach opens a way to select best embryo non-invasively for SET; thus, increasing implantation, while reducing multiple pregnancy complications.
Key words: *Morphokinetic, Time-lapse, Embryo selection.*

O-18
The investigation of transcript expression level of mitochondrial transcription factor A (TFAM) in single human oocytes during oocyte maturation

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Introduction: Impairment of human oocyte maturation during oocyte maturation is a cause of infertility in infertile women. Therefore, oocyte maturation is important in successful reproductive outcome of assisted reproduction technologies (ART). Mitochondria, which are the most organelle in the oocytes, have a critical role during oocyte maturation. Little is known about mitochondrial genomes during oocyte maturation. This study was to identify transcript expression level of mitochondrial transcription factor A (TFAM) gene, by using single-cell real-time PCR during human oocyte maturation.
Materials and Methods: 27 consenting women, aged 21-35 years, with male factors infertility were selected for ovarian stimulation and ICSI procedures. The mRNA level of the oocytes identified using single-cell taqman real-time PCR.
Results: There was a significant differences the relative expression levels of mitochondrial transcription factor A (TFAM) in stages of metaphase I (MI) and metaphase II (MII) oocytes as compared to germinal vesicle (GV) stage ($p<0.05$).
Conclusion: Human oocyte maturation is associated with the increased transcript expression level of nuclear (TFAM) encoded gene. Thus, any defect in the transcript expression level of nuclear transcriptional mitochondria (*TFAM*) gene leads to impaired developmental oocyte competence.
Key words: *Mitochondria, TFAM, Human oocyte, Taqman Real time-PCR.*

O-19
Cytoplasmic transplantation in oocytes obtained from ovarian tissue xenotransplantation lead to higher fertilization rate

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Introduction: Ovarian tissue cryopreservation and transplantation is one of the options which are used for fertility preservation in patient undergoing cytotoxic treatments such as chemotherapy. Xenotransplantation has been introduced as a reliable technique for better understanding of the transplantation conditions, but to achieve healthy embryos, the quality of obtained oocytes need to be improved.
Materials and Methods: Human to mouse dorsal muscle xenotransplantation technique was used for obtaining mature oocyte from cryopreserved human ovarian tissues. We investigated the capability of cytoplasmic transplantation in improving the oocyte quality in recipient oocytes (n=22).
Results: Cytoplasm transfer from healthy donor oocyte significantly improved the reanimation of the recipient oocyte quality, fertilization rate (76.5 vs. 40, $p<0.05$) and embryo quality.
Conclusion: Cytoplasmic transplantation after xenografting can be used for further exploration of the mechanisms involved in oocyte aging and poor developmental capacity of human ovarian oocytes in transplanted ovarian tissues.
Key words: *Human Ovarian Tissue, Xransplantation, Cytoplasmic Reconstraction.*

O-20
Reducing the risks in producing tissue engineered buccal mucosa as a bladder graft

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Introduction: Previous studies have shown that tissue engineered buccal mucosa has been used with good clinical outcomes in reconstructive surgery for the urethra. This involved the use of human acellular donor dermis, murine fibroblasts as a feeder layer to expand oral keratinocytes and bovine foetal calf serum to provide mitogens for these cells. Our aim was to avoid the use of donor human material and animal derived cells and sera in the production of tissue engineered oral mucosa to make it safer for clinical use. Our objectives accordingly were 1) to replace human donor dermis with a biodegradable electrospun polylactide scaffold to be used as a synthetic dermal alternative and 2) to replace mouse fibroblasts with screened human fibroblasts as a feeder layer and 3) to avoid the use of foetal calf serum.
Material and methods: 10% PLLA was used to produce an electrospun scaffold by electrospinning. The human fetal lung fibroblast cell line MRC- 5, used for more than 30 yrs in human vaccine production was used instead of murine 3T3 J2 fiboroblasts or oral fibroblasts were compared. In all cases oral keratinocytes were seeded into the scaffolds either in the presence or absence of FCS. Also, in separate experiments media was treated with bFGF or acid-2-phosphate to increase collagen production. The results were assessed using Alamar Blue for cell viability and Sirius red to assess collagen production on days 7 and day 14.
Results: Cells grew well on a 10% PLLA scaffold. We were able to expand oral keratinocytes in completely serum free conditions using either oral fibroblasts or MRC5 fibroblasts as a feeder layer. The presence of bFGF or ascorbic acid-2-phosphate also increased collagen production.
Conclusion: We have achieved several steps to produce TE buccal mucosa which does not require donor human tissue, murine feeder cells or bovine serum and thus presents less risk of viral disease transmission for the patient.
Key words: *Tissue engineering, Buccal mucosa, Bladder, Scaffold.*

O-21
Recurrent pregnancy loss causes and cures

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The etiology of recurrent pregnancy loss (RPL) is unknown in 30-50% of cases. Abnormal karyotype in partner, uterine and endocrine anomalies and immunological disorders are the most important clinical aspects that are recommended for identifying the cause of RPL. Thrombophila is one the most important single gene disorders that could cause recurrent pregnancy loss. However other single gene disorders also came in attention. Thyroid disease and Antiphospholipid Antibody Syndrome has been added to above category.
In total, 1247 couples with 2 or more consecutive pregnancy losses were admitted to Recurrent Abortion Research Clinic in Yazd Reproductive Sciences Institute participated in this study. Women were evaluated by obstetrics and gynecology consultant for anatomical problems in uterine. They also were evaluated for karyotype anomalies and endocrine disturbances. Semen analysis of the partners was evaluated and if there was a problem they referred to andrology clinic, and evaluations were done for urological anomalies and sperm chromatin assessment. Results of the research and treatment in this clinic showed that the most common causes were paraclinical hypothyroidism, which had a very good result for treatment and next success pregnancy. The least common causes were antiphospholipid syndrome, which treatment had a good result and majority of the women had success pregnancy but not all. Chromosomal abnormality was seen in 6% and mostly was in women showing aneuploidy of sex chromosome. Chromosomal polymorphism was seen in 12%, but the effect of it is still unclear. However the frequency of it is higher in RPL. Follow up of these families showed most of them had normal next pregnancy after visit with or without treatment.

Poster Presentations

P-1
Fertility preservation in cancer patients

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Introduction: Fertility preservation before gonadotoxic treatments is necessary in cancer patients. Ovarian tissue cryopreservation is the only available option for prepubertal girls and women who cannot delay cancer treatment or when ovarian stimulation is contraindicated.
Materials and Methods: Human ovarian tissue biopsies were obtained from six women (29-40 years old) who were candidates for oophorectomy for benign gynecologic conditions. Their medulla were removed, cortical parts were thinned and then cut into 10×5×1 mm strips. Cortical strips were vitrified in two steps by using vitrification solutions including; V1 [HTCM as base medium (BM)+ 7.5% DMSO+ 7.5% EG+ 20% HAS] and V2 (BM+ 15% DMSO+ 15% EG+ 0.25M sucrose+ 20% HSA) for 15 and 10 min respectively. Morphology of ovarian tissue and enclosed follicles were compared in control and vitrification groups by histological assessment.
Results: Ovarian stromal integrity, granulosa cells distribution, follicular population and morphology were well preserved in vitrified-warmed strips as fresh one. Oocyte degeneration, vacuolization and granulation were rarely observed in vitrified-warmed ovarian tissues. Primordial follicles were more distributed in cortical region in both groups. The mean percentage of intact follicles (primordial, primary and pre antral) are higher in control groups compared to vitrification one but the difference was not significant.
Conclusion: Accordingly, used vitrification method could be suitable for human ovarian tissue preservation in cancer patients.
Key words: *Human, Fertility preservation, Vitrification, Ovarian tissue.*

P-2
Effects of sesame-supplemented diet on the histology of adult rat reproductive system

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Introduction: Studies show that antioxidants are important in improving male infertility. There are several antioxidant compounds in sesame seeds.
Materials and Methods: This experimental study was carried out on 30 adults Wistar rat (200 gr). Rats were randomly divided into experimental and control groups. The control group received standard diet and experimental group received a diet containing 70% standard diet and 30% sesame seed for 12 weeks. At the end of the study, body weight, testis weight and volume were measured and histology of testis, epididymis and prostate were evaluated. Serum FSH, LH and Testosterone levels were measured as well. SPSS software was used to calculate t-test and $p<0.05$ was considered as significant.
Results: The measured sperm count and motility, the number of epithelial cells and lumen diameter were increased significantly in the experimental group ($p<0.0001$). The number of spermatogonial cells, primary spermatocytes, spermatid and spermatozoa were increased significantly in the experimental group compared to the control group ($p<0.0001$). The epididymal diameter, lumen diameter and epithelial thickness did not change significantly. Fibromascular and epithelium diameter of seminal vesicle in treatment group were very significant differences and volume density epithelium significantly increased in treatment group compared to control group but volume density, Fibromascular and lumen significantly decreased in treatment group compared to control group. LH concentration increased significantly in the experimental group ($p<0.03$).
Conclusion: This is the first study, which evaluated the histology effect of sesame seed on reproductive system of adult Wistar rat. These results highly suggest that sesame seed can improve male reproductive parameters.
Key words: *Rat, Male Sex hormones, Sesame seed, Testis, Prostate, Epididymis.*

P-3
The effects of sperm preparation media on motility, viability and DNA integrity of human spermatozoa

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Introduction: The main goal was to compare the effects of three different sperm preparation media on sperm motility, viability and DNA integrity of semen samples from normozoospermic men.
Materials and Methods: A total of 15 normozoo spermic males were included in the study. The semen analysis was performed in accordance with the WHO guidelines (2010). After semen analysis, each sample was divided into three aliquots and swim-up performed with three different sperm preparation media (Sperm Preparation Media, Origio, Denmark, Ham's F10, Biochrome, Berlin, Germany, and VitaSpermTM, Innovative Biotech, Iran). Sperm motility, viability and

DNA fragmentation,were evaluated at 0, 1, 2, and 24 hr after swim-up.

Results: There were no significant differences, at any time intervals, in the totalsperm motility between the different sperm preparation media. However, the rate of progressive motility was significantly higher in spermatozoa prepared by the media from Origio in comparison to VitaSperm TM (68.3±11.5 vs. 58±9.8, p=0.03), whereas no significant difference was found against Ham's F10. No significant differences in sperm viability were seen between the media products .One hour after swim-up therate of sperm DNA fragmentation was also significantly lower inthe medium from Origio versus VitaSpermTM (p=0.02).

Conclusion: The type of medium for preparation of semen samples from normozoospermic men significantly affects the performance of spermatozoain assisted conception.

Key words: *Sperm preparation media, DNA fragmentation (SCD), Viability, Motility.*

P-4

Investigating the underlying factors of preterm delivery prevalence preceded by preeclampsia among pregnant visitors of Shariati Hospital, Bandar-Abbas, Iran: 2011-2012

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Introduction: Along with blood loss and infection, disorders pertaining to blood pressure are the three deadly factors in preterm childbirth morbidities and disabilities. In other countries the prevalence rate of this crisis is maximally 10%, while in Iran, it is 7%.

Materials and Methods: The present descriptive/ analytic research is a cross-sectional retrospective study. It was carried out on 863 pregnant women (167 had a preterm delivery due to pre-eclampsia and 696 were diagnosed with preterm delivery due to other reasons). The data were collected via a checklist about mother's age, blood group, pre-eclampsia history, systemic disease, preterm delivery pain, etc. SPSS 16 analyzed the data. T-test and Chi-squared tests were used too. Significance level was set at $p<0.05$.

Results: Participants with a preterm delivery after pre-eclampsia were found to have a higher average age than peers afflicted with preterm delivery for other reasons. Most of the women afflicted with pre-eclampsia were of the B+ blood type. Significant differences were observed in these variables: pre-eclampsia history, preterm delivery pain, preterm rupture in fetal membrane, vaginal bleeding, intrauterine growth restriction, mother's systemic diseases, blood group, history of intrauterine death and amniotic fluid disorders.

Conclusion: There is an increase in the probability of preterm childbirth due to pre-eclampsia with intrauterine growth restriction, history of intrauterine death and mother's background diseases. Timely medical and preventive attempts and instructions for pregnant mothers on pre-natal healthcare can help to decrease irrevocable a consequence that endanger mothers and baby's health.

Key words: *Pre-eclampsia, Premature childbirth, Disease prevalence, Bandar-Abbas.*

P-5

Infertility and quality of life in women with polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS), the most common endocrine disorder, may cause infertility among affected women. Infertility is a life crisis and blamed for psychosocial distress and poor quality of life (QoL). Ethnicity and socio-cultural factors may have an impact on this negative influence on QoL.

Materials and Methods: The study procedure was completed by 796 women with polycystic ovary syndrome, aged 15-49 years. A reliable validated Persian version of the health related quality of life questionnaire (HRQoL) for polycystic ovary syndrome patients (PCOSQ) was filled for each participant. They were subdivided into 2 groups according to their fertility status: fertile and infertile. Using linear regression the association between infertility and health related quality of life in women with PCOS was assessed.

Results: Out of all participants 120 (15.1%) and 482 (60.6%) women were infertile and fertile, respectively. Infertility was associated with lower HRQoL score before and after adjustment for age, body mass index and other perceived PCOS symptoms (CI 95%: -16.76, -3.81; p=0.002 and CI 95%: -15.02, -3.67; p=0.001). Women who had longer duration of infertility, had got better scores.

Conclusion: We found that infertility causes poor quality of life in PCOS women. Care providers should pay attention to the effect of each PCOS symptoms on patients emotional health along with physical health and plan to treat them accordingly.

Key words: *Quality of life, Health-related quality of life, Infertility, Polycystic ovary syndrome.*

P-6

The effect of hydrostatic pressure on parthenogenetic activation of mouse oocytes

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Background: Parthenogenetic activation of mammalian oocytes using artificial stimuli is commonly used in various reproductive biotechniques. Hydrostatic pressure can act as a mechanical stimulator that rearranges egg contents. In this study, we investigated the effect of hydrostatic pressure on parthenogenetic activation of MΠ oocytes derived from superovulation and matured oocytes in vitro.
Materials and Methods: In experiment 1, immature oocytes were dissected from ovary of female NMRI mouse (8-week-old) and transferred to α-MEM medium for in vitro maturation. After 24 hr, MΠ oocytes were transferred to T6 medium. In experiment 2, mice were superovulated by injections of 10 IU of PMSG and 10 IU of HCG 48 hr apart. MΠ oocytes were collected 12 hr after HCG injection and transferred to T6 medium. Then oocytes from two groups (experiment 1, 2) were divided into experiment and control groups. Oocytes of experiment group were subjected to 20 mmHg pressure for 10, 20, 30 min (treatments I, Π, III). Oocytes without exposure to pressure were considered as control. Oocytes were cultured for 72 hr and embryo development was assessed.
Results: In experiment 1, cleavage rate in treatments I, Π, III and control was 21.42%, 15.71%, 8.52%, 5.71% respectively. The best cleavage rate were associated with treatment I which were significantly different with treatment III and control group ($p<0.05$). In experiment 2, cleavage rate in treatments I, Π, III and control was 22.91%, 53.12%, 29.15% and 9.34% respectively. Oocyte activation rate in experiment group was higher than control group. The highest cleavage rate associated with treatment Π which were significantly different with treatments I, III and control ($p<0.05$).
Conclusion: Exposure of MΠ oocytes derived from superovulation to hydrostatic pressure, on account of cumulus cells presence around oocyte, could improve embryonic development and by affecting on calcium channels, probably leading to increase rate of cleavage in the mouse oocyte**.**
Key words: *Parthenogenetic activation, Hydrostatic pressure, Oocyte, Mouse.*

P-7
Evaluation the knowledge and awareness of nulliparous women from adverse outcome of delayed childbearing

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Introduction: Recently the numbers of women who delay childbearing in older age were rapidly increased. The aim of this study was to evaluate the nulliparous women's knowledge and awareness from adverse outcome of delayed childbearing.
Materials and Methods: In this cross-sectional survey study, a total of 700 healthy nulliparous women 35 years and older that had self-choice delayed pregnancy were selected by cluster sampling in Tehran. To evaluate the knowledge and awareness of these women, a standard questionnaire about adverse pregnancy outcomes in older age were used. The questionnaire consists of three parts: demographic characteristics, assessment of maternal adverse outcomes (10 questions) and to assessment of fetal-neonatal adverse outcomes in maternal older age (6 questions). Data were analysis by SPSS software and the significance level was set at $p<0.05$.
Results: The psychometric properties of questionnaire including forward and backward translation, face and content validity and reliability with cronbach's-alpha: 0.078 were done. The result of the study showed that women's awareness about the risks of delayed childbearing including gestational diabetes, congenital anomaly, Down syndrome, caesarean section, low birth weight, stillbirth and long-term health problems like learning difficulties among neonate varies between 40%-72%. In this respect, most of the women were aware about the increased risk of infertility in older age (82.9%), but a little were aware about the possibility of a twin or multiple pregnancy and gestational hypertension in older ages of mother (35.2%). Awareness of delayed childbearing risks were independently related to the women's educational levels (OR: 1.87; 95% CI: 1.25-2.78) and family income (OR: 3.12, 95% CI: 0.01-5.12).
Conclusion: It seems that women are largely unaware about the adverse consequences of pregnancy in older age among mother and child. Women's should be more inform about these risks by health care professional.
Key words: *Awareness, Delayed childbearing, Adverse outcome.*

P-8
Study the protective role of jujube extract on teratogenic effects of Carbamazepine in Balb/c mice embryos

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Introduction: Carbamazepine (CBZ) is an anticonvulsant medication that is consumed during pregnancy and can produce congenital anomalies including; neural tube defects, cardiac, skeletal and craniofacial abnormalities.
Materials and Methods: In this experimental study, 100 pregnant mice of Balb/c 25±3 gr body weight randomly divided into 8 experimental groups (E) and 2 control groups (C) (n=10). Experimental groups (E_1, E_5 and E_6) and (E_2, E_7 and E_8) received daily

intraperitoneal injection (IP) of 50 and 100 mg/kg/ of CBZ respectively from gestational day zero (GD 0) to GD 15 and also groups (E_5, E_7) and (E_6, E_8) in addition to medicine, received jujube extract (AJE) at doses of 200 or 400 mg/kg/ from ten days prior to gestation, to GD 15. E_3 and E_4 groups gavaged only by 200 and 400 mg/kg of AJE respectively. Two control groups (C_1, C_2) received normal saline or tween -20. Dams underwent cesarean section on GD 18 and fetuses harvested from uterine. First, absorbed embryos were counted. Thereafter, morphological studies were done on the offspring by stereomicroscope. All malformed fetuses were stained with Alizarin red S and alcian blue for detection of skeletal anomalies. Data were analyzed by ANOVA, Tukey and χ^2 tests and using SPSS software version 18 and $p<0.05$ were considered significant.

Results: The findings of this study showed that administration of CBZ purely in pregnant mice induced various anomalies in their fetuses such as; limb defects, deformities of vertebral column, craniofacial malformations and etc in experimental groups so that their differences were significant when compared with control groups. But these anomalies decreased significantly in those experimental groups which received CBZ and AJE synchronously when compared with experimental groups that received CBZ only.

Conclusion: According to our findings it can be concluded that although administration of CBZ can induce several malformations in fetuses of pregnant mice, but consumption of AJE synchronously with CBZ, can prevent teratogenicity of CBZ. Therefore, probably AJE can play a protective role against CBZ induced anomalies.

Key words: *Teratogenic, Jujube, Carbamazepine.*

P-9

Depression and clinical markers in polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive-age women. Previous studies have raised conflicting results of depressive disorders in PCOS compared with healthy people.

Materials and Methods: In this cross-sectional analytic study, 62 patients with PCOS diagnosed by Rotterdam criteria and 61 women without PCOS were selected after an initial survey conducted to determine demographic characteristic, systolic and diastolic blood pressure and reproductive status, the Beck depression questionnaire short form was used to assess depression in both groups. All women were introduced to determine fasting insulin and blood sugar and testosterone.

Results: The mean age, BMI, systolic and diastolic blood pressure of PCOS and Non PCOS was respectively (29.96±6.85, 29.49±7.44 years), (29.15±6.56, 25.65±5.84), (117.42±10.17, 117.18±66.57) and (75.41±9.27, 76.42±6.56) respectively that only mean of BMI was different ($p<0.05$). The average Beck score in patients was 7.47±5.54 and in the control group was 7.57±5.79, ($p<0.05$), which was not statistically significant. 37.1% of the patient group and 36.1% of the control group have degree of moderate to severe depressed mood ($p>0.05$).

Conclusion: Despite non significant difference in prevalence of depression in both groups, due to the high prevalence of depression in both groups may be there is other reasons for depression in this population that overcomes on the psychological effects of this disease.

Key words: *Depression, Polycystic ovary syndrome, BMI.*

P-10

Comparison of metabolic syndrome in postmenopausal women with natural or surgical menopause: Community based cohort study

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Introduction: The ovaries produce female sex hormones that after natural menopause gradually and following surgical menopause abruptly end.

Materials and Methods: The study subjects were selected from 5019 women, 59-35 years old, which were participated in the Tehran Lipid and Glucose Study. They include 357 natural menopauses and 63 surgical menopauses during the follow-up that was 10 years. Data were collected by questionnaires in this project.

Results: Changes in metabolic and biochemical profiles of these two groups of women during follow-up were compared with each other. The findings of our study showed that the incidences of metabolic syndrome in surgical menopause and natural menopause subjects were 14.5 and 8.9% respectively. Mean serum low density lipoprotein in surgically menopausal women was significantly lower than natural menopause subjects. Also, mean systolic blood pressure in naturally menopausal women was significantly higher than surgical menopause women.

Conclusion: It seems the metabolic disorders associated with menopause differ in surgical and natural menopause women. The metabolic complications should be considered following menopause.

Key words: *Surgical menopause, Natural menopause, Metabolic syndrome.*

P-11
Medical staffs' viewpoints on "ART nursing" working at Reproductive Institutes in Iran

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Introduction: As, assisted reproductive techniques (ART) advances, multifaceted changes are noted in the roles, responsibilities, and commitments of each members of the infertility team. The aim was to determine the level of knowledge and attitude of medical staff working at reproductive institutes about role of nursing in ART program.
Materials and Methods: In this descriptive cross-sectional study, knowledge and attitudes of 199 Bio-medical staffs working at reproductive institutes were investigated through a questionnaire including 20 questions. All participants based on work experience, education and communication with patients were classified. Chi-square test was used for data analysis and $p<0.05$ was considered as statistically significant.
Results: There was no significant relationship between educational levels and viewpoints on ART nursing. But, significant relationship between work experience, kind of relation to the patients and viewpoints on ART nursing were found. 52.5% of participants were familiar with ART nursing. By increasing experience, knowledge and attitudes of Bio- medical staffs enhanced. Laboratory technician, embryologist, ART Laboratory technician and nurses were more familiar with ART nursing; but, Urologists were less familiar with ART nursing. Over 97% of participants requested offering a master degree of ART nursing in Iran ($p<0.05$).
Conclusion: There is a need for nursing professionals to assume an ongoing, visionary, scientific, and academic approach to advancement. It seems that appropriate continuing education and opportunities are essential to support this group of nursing professionals.
Key words: *Viewpoint, Nurse, ART, Medical staffs.*

P-12
The effect of microtubule stabilizer pretreatment in IVM program

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Introduction: It has been reported that in vitro maturation (IVM) increases the risk of abnormal spindles and chromosome configurations of oocytes compared with oocytes matured in vivo. This is one possible explanation for the reduced developmental potential of IVM oocytes compared with those matured in vivo. Paclitaxel is known to stabilize the microtubules that constitute the spindle. The aim was to investigate whether pretreatment by a microtubule stabilizer, paclitaxel, would improve IVF outcomes in IVM program.
Materials and Methods: In this ongoing experimental study, 75 GV oocytes were retrieved from 5 mice primed with rec-FSH for 48 hr. The immature oocytes were cultured 24-48 hr for IVM. After identifying mature oocytes by the presence of a first polar body extrusion under a stereomicroscope, Normal morphologically MII oocytes were divided in control and 5 experiment groups. Experiment groups were incubated in presence of 1μM paclitaxel for different times (30 min/group 1,1 hr/group 2,2 hr/group 3,3 hr/group 4,4 hr/ group 5 and group 6 as control) prior to IVF. Standard IVF program was performed for the 60 MII oocytes after pretreatment with paclitaxel and embryo development was followed until blastocyst formation.
Results: Total maturation rate of GV oocytes was 80% and fertilization rates of MII oocytes were 85% in groups 6, 83% in groups 1, 2 and 3 and 74% and 65% in groups 4, 5 respectively. Blastocyst formation rates of embryos were 95% in group 1, 2 and 6, 60% in group 3 and 40% in groups 4 and 5. The final results, which include probable relationship between paclitaxel pretreatment and the aforementioned variables, will be presented.
Conclusion: The preliminary results showed by performing critical concentration with optimized timing of microtubule stabilization procedure IVM, MII oocytes could efficiently develop to the blastocyst stage. Paclitaxel may cause cell toxicity with irreversible harm on normal fertilization and embryo development in higher concentrations.
Key words: *Paclitaxel, IVM, IVF outcomes.*

P-13
The effect of ovarian drilling on poly cystic ovary syndrome (PCOS) women

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Introduction: One of the most common causes of female infertility factors is ovulation disorder. Poly cystic ovary syndrome (PCOS) has the highest prevalence rate about 5-10% among women. There are different diagnostic methods and treatments for PCOS syndrome, including medical and surgical treatments. Laparoscopic surgical treatment is for the patients resistant to medical treatment, and could be done with ovarian drilling at some points.

Materials and Methods: In this retrospective study we evaluated 289 patients. All patients had proven clomiphene resistant PCOS with ultrasound and hormonal tests. Laparoscopic ovarian cautery was performed for all these women. Age, duration of infertility and assisted reproductive techniques in each patient were asked.
Results: Of the 289 patients, we access to the information of 135 patients. The mean age and the duration of infertility were 26.90 and 4.83 years respectively. Among these, 63 patients (46.6%) had not pregnancy and 72 patients (53.33) were pregnant, 52 patients (72.22%) were naturally pregnant and 20 patients (27.77%) used assisted reproductive techniques.
Conclusion: PCOS patients who resistant to clomiphene could be treated with ovarian drilling.
Key words: *Polycystic ovary, Clomiphene, Ovarian drilling.*

P-14
Comparison of two different embryo loading techniques for embryo transfer in IVF/ET cycles

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Introduction: Embryo loading (EL) is one of main steps in embryo transfer (ET) technique that plays an important role in IVF success. This study was aimed to compare the effect of two different techniques for EL on rates of pregnancy and delivery in IVF/ET cycles.
Materials and Methods: In total 195 fresh ET and 171 frozen-thawed ET were included in this retrospective study in which 76 and 119 cycles in fresh ET and 76 and 95 cycles in frozen-thawed ET were placed in two groups of A and B, respectively. Embryo catheter loading techniques were divided into two groups of A and B. In group A, the whole catheter was flushed with a 1-ml air-tight syringe with Ham's F10 medium. Then the embryos were drawn into ET catheter between two air brackets. In group B, 70 µl airs was held in the syringe and the catheter was flushed by Ham's F10 medium. Then the media, air, embryos, and air were respectively drawn into catheter. The main outcome measure of the study was delivery rate.
Results: The groups were matched for number of fertilized oocytes, etiology of infertility, source of sperm, type of stimulation protocol, percent of conventional IVF or ICSI, high quality embryos, type of embryo loading catheter, fresh ET and ease of transfer. In fresh ET cycles, delivery rate showed an increasing trend in group B compared to group A (21% vs. 11.8%, respectively, p=0.1). In frozen-thawed ET cycles, the rate of delivery rate was also higher in group B compared to group A (16.8% vs. 13.1%, respectively), but the difference was not significant (p=0.5).
Conclusion: It seems different embryo catheter loading has no effect on delivery rate.
Key words: *Embryo loading technique, Embryo transfer, Pregnancy.*

P-15
Application of sonography in infertility treatment cycles: instructions for midwives and nurses

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Introduction: Ultrasound has a pivotal role in imaging modality in the study of the female pelvis, and provides fundamental information in detecting and characterizing pelvic masses of uterine, ovarian, or adnexal origin.
Materials and Methods: A narrative review was performed within articles published at PubMed, Elsevier, SID and original text books to reach the aim.
Results: Every patient in the process of infertility treatment needs to go through four steps of sonography to roll out disorders and malformations in ovaries, uterus and tubes then to opt for the best treatment method and to follow up the results. Infertility Workup: 1) Base sonography is applied in examination of the following organs: -For overall evaluation of the pelvis and determining any pathologic condition in uterus such as polyps, fibroms, uterus malformations. -Ovarians are examined for ovarian cysts and Poly Cystic Ovary Syndrome (PCOS). 2) Pre-treatment sonography is applied in checking the result of operative surgeries-like Hysteroscopy, Laparoscopy, Myomectomy. 3) Monitoring sonography is done at different stages of treatment cycle, especially at ovulation stimulation period, in order to investigate ovarians and in picking up the eggs as well as endometrial thickness in response to drugs. 4) Post-Assisted reproductive techniques (ART) cycles is done for confirmation of pregnancy (gestational sac, fetal heart), and the side effects of treatment cycles such as ovarian hyper stimulation syndrome (OHSS), pregnancy complications such as ectopic pregnancy (EP) and mole.
Conclusion: Ultrasound has a key role in diagnosis and proposing of different options of treatment regimes, and the post-treatment follow-ups. It is important to know the application of the aforementioned four steps to improve the accuracy and efficiency of infertility treatment.
Key words: *Ultrasonography, ART cycle, Infertility.*

P-16
Evaluation of anxiety, depression and risk factor associated with them in women after tubal ligation

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Introduction: Tubal ligation is recommendable for women completed their family. The existence of anxiety and depression following this procedure has been the subject of debate for decades.
Materials and Methods: A historical cohort study was carried out on 200 subjects with tubal ligation and on 200 subjects using condom as contraceptive method. The two groups were matched in demographic and personal characteristics. Data collection tool was a questionnaire including questions regarding demographic and obstetrical characteristics. Anxiety and depressive symptoms were evaluated by the Hospital Anxiety and Depression Scale (HADS). All statistical analyses were carried out using software package used for statistical analysis (SPSS) version 20 (SPCC Inc., Chicago, IL, USA). Student's t-test and chi-square test were carried out to reveal the statistical differences between the groups. Multiple linear regression was done to build a prediction model in anxiety and depression.
Results: The mean (SD) duration of tubal ligation was 4.1 (1.6) years. The overall prevalence of anxiety and depression in the two groups was 81.5% and 48.5% respectively ($p<0.0001$). Multiple linear regression analysis revealed a significant association between low education ($p<0.0001$), post-sterilization regret ($p=0.03$) and no consultation prior tubal ligation ($p=0.03$) with risk for anxiety and depression in women who have undergone tubal ligation.
Conclusion: Termination of fertility with tubal ligation may be a risk factor for anxiety and depression. We found significant differences in anxiety and depression between women with and without tubal ligation. Therefore, women should be informed by the health providers regarding the advantages and disadvantages of tubal ligation before the procedures.
Key words: *Historical cohort study, Tubal Ligation, Anxiety, Depression.*

P-17
Polymorphism in *CGA* affects the function of miR-1302 and increases the risk of men infertility

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Introduction: Infertility occurs in 10-15% of couples worldwide and close to half of it is caused by male factors. Despite decades of efforts to clarify mechanism of male infertility, most of cases are still idiopathic. Many factors such as genetics and sexual problems can affect infertility. Among these problems, genetic disorders are the most common factors. A study has shown that one of genes that can affect male infertility is *CGA*. This gene is involved in miotic. CGA, α subunit of glycoprotein hormones, is the main part of thyrotropin glycoprotein hormone (pituitary TSH), lutropin (LH), follitropin (FSH), and Chorionic gonadotropin (human placental gonadotropin, hCG) that has essential role in development and function of tyroid and gonads. *CGA* gene is located on 6q14-q21. Rs6631 in *CGA* has strong association with men infertility. Studies have shown that miR-1302 can negatively regulate *CGA* and substitution of T with A may interfere this process. This miRNA can band with rs6631-A more strongly than rs6631-T.
Materials and Methods: Tetraprimer technique is an appropriate way to study this polymorphism; because it is faster and cheaper than ordinary PCR. Also laboratories with low equipment can use this method.
Results: Primers designed by the use of Primer1 and then checked by Oligo7 software. By the use of these primers and tetraprimer technique, this polymorphism can be studied for the first time in Iran.
Conclusion: SNP in genes specially the ones that are target of miRNAs, can have important role in complex disease such as infertility. In this study, miR-1302 negatively regulates *CGA*, and the substitution of T by A at rs6631 within the binding site disrupts its regulation. It is not exactly clear that what is the real role of this polymorphism in idiopathic male infertility; but it has been suggested that the variant allele of rs6631 may elevate the risk of idiopathic male infertility through up-regulation of the expression of CGA. It has been showed that α-subunit, that is produced by CGA, can has growth factor activity and can induce the differentiation of lactotrope and secretion of RPL. Because CGA and PRL play an important role in the development of gonads, the abnormal hormone level may elevate the risk of male infertility.
Key words: *Men infertility, miRNA, SNP, CGA.*

P-18
Evaluation of testosterone levels after GnRH agonist administration in the adult male rat

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Introduction: Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus in a pulsatile manner and stimulates the biosynthesis and release of gonadotropins, LH and FSH, via GnRH receptor located in gonadotropin cells. These gonadotropins regulate

various gonadal functions such as gametogenesis and steroidogenesis.

Materials and Methods: 24 male adult Wistar rats were divided into three groups. In the first study group, 300 µg/kg buserelin, in the second study group, 600 µg/kg buserelin and in the control group, salin was injected subcutaneous for 5 days. Thirty day after the first injection, blood samples were collected from the heart, centrifuged and plasma was isolated from blood. Plasma testosterone level was measured by ELISA.

Results: The findings reveal no significant differences in plasma level of testosterone in the first study group (2.71±0.71) and second study group (2.12±1.74) compared with control group (3.14±1.20) ($p>0.05$).

Conclusion: Short-term administration of buserelin has no effect on the plasma testosterone level.

Key words: *GnRH, Buserelin, Testosterone, Adult rat, ELISA.*

P-19

Apoptotic cells and loss of follicle development were resulted after administration of Nano dioxide titanium on immature mouse ovary

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Introduction: Titanium dioxide (TiO_2) is used as an antimicrobial and whitening agent in food and products such as chewing gums, candies, toothpastes, lip balms, shampoos, deodorants and sunscreens. Previous studies showed that ovary might be one of the target organs that TiO_2 nanoparticles induced genotoxicity and cytotoxicity in ovary cells and exposure to TiO_2 can effect on follicle development and fertility with changes the levels of sex hormones. The aim of the present study was to evaluate ovarian dysfunction by detect of follicle development.

Materials and Methods: 40 immature Balb/C female mice (4week ages) randomly divided into five groups. First, second and third groups were administrated by oral gavage 2.5, 5 and 10 mg/kg TiO_2 for thirty days respectively. Forth group was gavaged normal saline as placebo and in control group we didn't gavage anything. Finally female mice were dislocated and ovary was fixed in bouin's fluid. Ovaries were dehydrated with ethanol series, embedded in paraffin and then serially sectioned at 5µm thickness. Hematoxylin and Eosin used to evaluate primordial, primary, preantral and antral follicles by light microscope. TUNEL assay was used to detect cell apoptosis. Statistical significance was analyzed by Kruskal-Wallis test and the level of significance was determined to be at $p\leq0.05$.

Results: According to data, primordial, primary, preantral and antral follicles were significantly decreased in third group in comparison with the controls and shams ($p\leq0.05$) and also, there was increasing trend in primary and preantral follicles in first and second groups compared to control group during TiO_2 exposure. Although, significant reduction of apoptotic cells was observed in third group compared to control and sham groups ($p\leq0.05$).

Conclusion: Furthermore, there is little knowledge about physicochemical characteristics of TiO_2 nano materials; our findings suggested that TiO_2 exposure can defect follicle development and perhaps fertility potential in young mice.

Key words: *Titanium dioxide, Ovary, Follicle development, TUNEL.*

P-20

Correlation between 25-OH vitamin D in follicular fluid and implantation rate in infertile women undergo IVF/ICSI

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Introduction: Vitamin D in combination with its receptors can be involved in implantation by regulating gene expression, endometrium immune response and stimulation of endometrium decidualization. So, it seems that the amount of vitamin D in follicular fluids (FF) may has an association with ART success.

Materials and Methods: In a prospective study, 80 infertile female candidates for IVF/ ICSI were enrolled. Blood samples (on the day of human chorionic gonadotropin) and follicular fluids were taken, and then levels of serum estradiol and follicular fluids 25-OH vitamin D were measured. Also clinical characteristics of patients (duration of infertility, causes of infertility, and menstrual status), number and quality of oocytes, number of fertilized oocytes, estradiol levels, and clinical pregnancy were evaluated.

Results: Concentration of FF 25-OH vitamin D in pregnant women was significantly higher than non-pregnant women ($p=0.007$) but there were no significant differences in age, body mass index (BMI), duration of infertility, menstrual status, number of oocytes, oocytes quality, number of fertilized oocytes and serum estradiol levels between the two groups ($p>0.05$). Statistically positive correlation was found between 25-OH vitamin D levels with patient age and implantation rate ($r=0.264$, $p=0.018$ and $r=0.301$, $p=0.007$ respectively).

Conclusion: The obtained results suggest that vitamin D without affecting the number and quality of oocytes can independently improve implantation rate and IVF outcome.

Key words: *Embryo implantation, Vitamin D, IVF outcome.*

P-21

The protective effect of N-acetyl-L-cysteine on spermatogenesis and sperm characteristics in mice following exposure to Para-Nonylphenol

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Introduction: Para-Nonylphenol (p-NP) is an environmental contaminant with wide industrial applications which causes oxidative stress in different organs such as the reproductive system. The purpose of this study was to investigate the protective effect of N-acetyl-L-cysteine (NAC), as a powerful antioxidant, on spermatogenesis indices, sperm parameters, chromatin quality and tail length following treatment with para-Nonylphenol in adult mice.
Materials and Methods: 24 adult male NMRI mice (32±4 gr) were divided randomly into 4 groups (n=6), control, NAC (150 mg/kg/day), p-NP (250 mg/kg/day) and p-NP+NAC, and they were treated orally for 35 days. By the end of the treatment, mice were weighed and sacrificed, Their right testis was also weighed then the left caudal epididymis was cut in Ham's F10. Released spermatozoa were used to analyze the motility, viability and the abnormalities of the sperm. The sperm tail length was estimated by stereological methods. Sperm chromatin quality was also assessed by nuclear staining using acridine orange and aniline blue dyes.In continue, the right testis were taken out, fixed, sectioned, processed and stained using heidenhain azan method. Spermatogenesis indices including the tubular differentiation index (TDI), Sertoli cell index (SCI) spermatogenesis index (SI), meiotic index (MI) and repopulation index (RI) were studied. Data were statistically analyzed using one way ANOVA and means were considered significantly different at $p<0.05$.
Results: A significant decrease in the spermatogenesis indices, sperm motility, viability, sperm tail length and the number of sperms with normal morphology was observed in the p-NP group compared with the control ($p<0.002$), while no significant change was found in the sperm chromatin quality. The above parameters significantly increased in the p-NP+NAC group compared to the p-NP treated ones ($p<0.01$).
Conclusion: N-acetylc-L-ysteine, as an antioxidant, can prevent the adverse effects of para-Nonylphenol exposure on spermatogenesis indexes and sperm parameters in mice.
Key words: *Para-Nonylphenol, N-acetyl-L-cysteine, Spermatogenesis indices, Sperm parameters.*

P-22

The effect of vitamin C on human vitrified sperm parameters in normozoospermic men: raw semen and washed semen

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Introduction: Vitamin C is a common component in seminal fluid. It can improve sperm parameters as an antioxidant. The objective was to evaluate the effect of Vitamin C on human vitrified sperm parameters in normozospermia men.
Materials and Methods: Semen samples were collected from 40 normozoospermic samples and divided into 4 groups. Group 1, raw semen was vitrified. Group 2, semen processed by swim up method and then vitrified. Group 3, vitamin C (600 µm) was added to raw semen and then vitrified. Group 4, vitamin C (600 µm) was added to prepared spermatozoa and then vitrified. The semen analysis was performed according to WHO criteria before and after vitrification.
Results: In this study data showed that progressive motility and immotile spermatozoa after vitrification were significantly different between groups ($p=0.014$, 0.005 respectively). All groups were compared, and the results revealed that progressive motility and immotile spermatozoa after vitrification were statistically significant in washed semen+vitamin C group compared with wash semen and raw semen groups ($p=0.05$, 0.01 for progressive motility and $p=0.04$, 0.007 for immotile spermatozoa respectively).
Conclusion: Adding vitamin C, as an antioxidant, to washed semen has shown beneficial effect on sperm motility after vitrification.
Key words: *Sperm parameters, Vitamin C, Vitrification.*

P-23

Review of fertility diet and its effect on infertility among women from the viewpoint of Iranian traditional medicine in comparison with modern medicine

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Introduction: In the recent years the infertility diagnosis and treatment methods have undergone a radical change and development but still, the success rate of modern treatments like in vitro fertilization (IVF) even in the best centers is between 30-40%.

Materials and Methods: The available texts on the Iranian traditional medicine were reviewed. Besides, the relationship between fertility diet factors and infertility among women was investigated through reviewing databases on the world-wide web.
Results: Iranian traditional medicine investigates the relationship between nutrition and infertility from a perspective postulating that complying with fertility diet principles helps maintain healthiness and improve fertility. From this view, body members have their special temperaments; if balanced, body members function normally. Any kind of abnormal temperament happened to the semen or uterus results in infertility or hardship in fertility. These are seen as disorders in sanguine, choleric, melancholic, and phlegmatic temperaments. It should be noted that the bad temperaments are mainly caused by not following the health and fertility diet.
Conclusion: The different viewpoint of the Iranian traditional medicine towards the relationship between infertility and nutrition and its focus on the nature of food and nature of a person could be of interest to researchers in the field and if approved, help to prevent infertility or treat infertile couples.
Key words: *Infertility, Iranian traditional medicine, Infertility diet.*

P-24
The in vitro fertilization outcome and luteal phase GnRH antagonist administration

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Introduction: Genital tissues (ovary, endometrium and placenta) express GnRH receptors. GnRH plays essential roles in embryo implantation, invasion of trophoblastic tissue and steroid synthesis in the placenta. In IVF-ICSI cycles, the use of GnRH antagonists is limited to the last days of ovulation. The aim of this study was to evaluate the effects of GnRH antagonist at pharmacological doses given in the early implantation period on pregnancy.
Materials and Methods: This retrospective study was performed in Yazd Research and Clinical Center for Infertility, 2014-2015. Women under 40 years old, with >20 follicles (>11mm) and risk of OHSS were included. Participants with history of endometriosis, hysteroscopy and history RIF were excluded. The treatment for all of participants was antagonist protocol. Twenty seven patients did not receive Cetrotide in luteal phase, and 67 patients received Cetrotide. Pregnancy outcomes were assessed based on chemical and clinical pregnancy.
Results: Totally 94 patients were included. The mean age of participants was 28.40±4.25 years old. There were no differences in ART and demographic characteristics ($p>0.05$). The most frequent causes of infertility were: male factor (45.2%) and polycystic ovary syndrome (19.4%). The pregnancy outcome was not significantly different between Cetrotide and non Cetrotide group ($p=0.224$).
Conclusion: The present study proposed that luteal phase GnRH antagonist administration does not influence the chance of successful pregnancy outcome. The incidence of chemical and clinical pregnancy in two groups was not significantly different.
Key words: *GnRH antagonist, Pregnancy outcome, IVF.*

P-25
Hazardous effect of acrylamide on development landmarks in rat offspring and the role of Glycyrrhiza Glabra

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Introduction: Acrylamide (AA) is a chemical substance used mainly in certain industrial process. Monomer of Acrylamide formed in food stuffs containing carbohydrates during high temperature cooking.
Materials and Methods: 24 pregnant rats were selected for this study. The pups of these mothers divided into four groups: group A:(control group); group B: (ACR administration 10 mg/kg/day orally); group C: (GG administration 150 mg/kg/day orally); group D: (ACR+GG). At day 21 the pups were evaluated for developmental study. The results were analyzed by SPSS software (15) and $p<0.05$ was significant.
Results: The result demonstrate that eye opening and fur development appeared occurred slightly later in AA administration group.
Conclusion: These results showed that AA treatments induce delay in developing offspring and GG as an antioxidant reduce these changes.
Key words: *Acrylamide, Glycyrrhiza glabra, Eye opening, Fur appearance, Offspring.*

P-26
The effect of long-term exercise via TNF-α level on the type-1 diabetic rats in testis tissues

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Introduction: Approximately 90% of diabetes patients often show sexual abnormalities and impotence infertility. Damages caused by hyperglycemia and oxidative conditions result in inflammatory mediators increasing such as cytokines in tissues of sex organs. The TNF-α is the first locally produced cytokines in chronic inflammation pathway. TNF-α level in diabetics blood is higher than normal people that probably histological studies of TNF-α could determine source of this increasing. Also exercise can reduce complications of diabetes especially in tissue by reducing of oxidative elements and establishing of Hypoglycemia.
Materials and Methods: In this study, 40 male rats weighing 200-250 gr were used and randomly were classified into four groups; each group consist 10 rats (control, diabetic, healthy with period of 60-day exercise, diabetic with period of 60-day exercise). Treadmill exercise daily was 1 hr with 22 m/min speed. Diabetes was induced by injection intraperitoneal of streptozotocin with the amount of 60 mg/kg. After expelling of the testis under general anesthesia, samples were homogenized and TNF-α protein levels were measured according to ELISA kit (special rat TNF-α).
Results: The level of TNF-α showed no significant changes among diabetic groups in testicular tissue compare to control groups. Also, exercise didn't have any negative effects on the level of TNF-α in exercise control group compare with control group. Therefore, we didn't show any significant change of TNF-α level between our groups.
Conclusion: In this study, the period of disease time may be is not enough to determine the level of TNF-α via exercise treatment in testis tissues. It seems that duration of diabetic developing, severity and type of exercise could be many important reasons to change inflammatory mediators.
Key words: *Cytokine, Hyperglycemia, ELISA Kit, Sexual dysfunction.*

P-27
Evaluation of progesterone and estradiol levels after GnRH agonist administration in the adult female rat

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Introduction: Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus in a pulsatile manner and stimulates the synthesis and release of gonadotropins, LH and FSH, via GnRH receptor located in adenohypophysis. These gonadotropins, regulate various gonadal functions such as folliculogenesis and steroidogenesis. Various data are reported of GnRH agonists effects on ovary steroidogenesis.
Materials and Methods: 24 female adult Wistar rats were divided into three groups. In the first study group, 300 µg/kg buserelin, in the second study group, 600 µg/kg buserelin and in the control group, the same amount of salin was injected subcutaneous for 5 days. 30 days after the first injection, blood samples were collected from the heart, centrifuged and plasma was isolated from blood. Plasma esteradiol and progesterone levels were measured by ELISA.
Results: The findings reveal a significant incereas in plasma level of estradiol in the second study group (165±104.89 pg/ml) in comparison with control group (55.47±50.32 pg/ml) and in the second study group compared with the first study group (47.28±32.95 pg/ml) ($p<0.05$). But no significant differences were observed in plasma level of progesterone in the three groups ($p>0.05$).
Conclusion: Short-term administration of high doses buserelin increase plasma estradiol level, while has no effect on the plasma progesterone level.
Key words: *Buserelin, Progesterone, Estradiol, Adult rat.*

P-28
A comparative study on the effect of face-to-face or group education during the pregnancy period on sexual function of the couples under coverage of selected clinics in Isfahan in 2013

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Introduction: Pregnancy can conflict with sexual function that can be affected by physical and psychological changes during Pregnancy.
Materials and Methods: In this quasi-experimental pre-post-test study, 64 couples with pregnant women were selected and randomized in two groups in Isfahan. The data were collected via the triangulation of FSFI, BFSI and demographic characteristics questionnaires. SPSS -18 was used to analyze the data by descriptive and inferential statistics.
Results: No significant difference was found in the demographic characteristics between two groups. Education was effective on sexual function in two groups of women ($p<0.001$), but no significant difference was found between two groups ($p=0.61$). Also education was effective on sexual function of the men in both groups ($p<0.001$), and there was a significant difference between the two groups ($p=0.003$). Meanwhile, there was no significant difference between couples regarding the education ($p=0.104$).
Conclusion: The results of the study showed that type of education plays a role in improvement of sexual function in pregnancy. In addition, sex education is effective on prevention of sexual disorders in

pregnancy. Therefore, having a special approach toward sex education classes during pregnancy is inevitable for the health providers, particularly midwifery professionals.

Key words: *Group education, Face to face education, Sexual function, Pregnancy, Iran.*

P-29

Assisted reproductive technology outcomes in couples with hepatitis virus infection

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Introduction: Currently, many hepatitis positive infertile couples attend assisted reproductive technology (ART) program, so there is concern as this viral infection may be deleterious to human fertility. Some have suggested that hepatitis virus have an adverse effect on pregnancy outcomes.

Materials and Methods: The study was conducted in two groups of patients: A) seropositive for hepatitis B virus (HBV) and hepatitis C virus (HCV) (n=46); and B) patients lacking these viruses as a control group (n=32). Patients included couples seeking ART from January 2010 to October 2014. ART cycle characteristics and clinical outcomes were assessed between two groups.

Results: Demographic characteristics and age were similar between two groups. The mean±SD age was 30.19±5.54 and 34.31±6.86 years in female and male, respectively. Regarding to oocyte number and embryo score, there was no significant differences between groups A and B. The rate of pregnancy was, however, significantly higher in group B in comparison with A (p=0.03).

Conclusion: Although, the data on embryo formation was similar in both groups of infertiles, but the pregnancy outcomes were noticeably reduced in infertile patients with hepatitis virus infections.

Key words: *Hepatitis virus infection, ART, Pregnancy outcome.*

P-30

The influence of the Corpus Luteum (CL) on hormonal and biochemical metabolites composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows

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Introduction: Metabolic changes in blood serum may be rejected in the biochemical composition of follicular fuid (FF) and could indirectly influence oocyte quality. In addition, the levels of hormonal and biochemical metabolites in the FF were related to follicular size and interestingly to the presence or absence of a corpus luteum. The purpose of this study were to examine the influence of the corpus luteum on hormonal and biochemical metabolites composition of follicular fluid (FF) harvested from different sized follicles and their relationship to blood serum in dairy cows.

Materials and Methods: Ovaries were recovered from 30 female adult cows (Holstein Friesian) 4-7 years of age with clinically normal reproductive tracts after slaughtering. Blood samples were collected from the jugular vein before slaughter from each cow. The stage of the cycle in the cows slaughtered was diestrus determined post mortem. The ovaries collected per cow were classified with corpus luteum (CL+) and without corpus luteum (CL-). Visible follicles on the surface of the ovaries were classified, based on their diameter, into (I) small (3-5 mm), (II) medium (6-9 mm) and (III) large (10-20 mm) categories. Follicular fluid was aspirated from different sized follicles in CL+ and CL- ovaries. Serum and FF samples were analyzed for hormones (estradiol-17β, progesterone, testosterone, T3 and T4) and biochemical metabolites (glucose, cholesterol, triglyceride, total protein, albumin and globulin).

Results: Results showed that the FF concentration of estradiol-17β, progesterone and testosterone in different size follicles categories (small, medium and large follicles in CL+ and CL- ovaries) were significantly higher ($p>0.05$) when compared with the serum. The FF concentration of estradiol-17β, testosterone, glucose and cholesterol in same follicle size categories of CL- ovaries were significantly higher ($p>0.05$) when compared with CL+ ovaries. In the present study, the serum concentration of glucose, cholesterol and triglyceride were significantly higher ($p>0.05$) when compared with the fuid from different sized follicles categories in CL+ and CL- ovaries. The differences between follicle size categories in CL+ ovaries were only significant for concentration of estradiol-17β and in CL- ovaries were significant for concentrations of estradiol-17β, glucose and triglyceride.

Conclusion: According to the results of the present study, the levels of the biochemical metabolites in the FF were related to follicular size and interestingly to the presence or absence of corpus luteum.

Key words: *Dairy cow, Follicular fluid, Corpus luteum, Hormone, Metabolite.*

P-31

Health related quality of life in women with polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS) is associated with symptoms that affect psychological wellbeing and health-related quality of life (HRQoL).

Materials and Methods: This was a retrospective case-control study which was conducted on 116 women from April-August 2014 in Rasht, Iran. Cases were 60 newly diagnosed women with PCOS, according to the Rotterdam criteria while the controls were 60 healthy women and groups were matched on sociodemographics characteristics. Data needed to determine health-related quality of life were collected via convenience sampling using modified PCOS quality-of-life questionnaire (MPCOSQ) that contains 6 subscales: emotional disturbances, hirsutism, infertility, weight, menstrual and acne. Data collected were analyzed using statistical T-test, chi square and Kolmogorov-Smirnov.
Results: Finding demonstrated that groups were matched in age, body mass index, marital status, occupation, educational status and socioeconomic status. The mean HRQoL score was significantly lower among women with PCOS (20.96±7.4) than among controls (29.8±4.5) ($p<0.001$). The mean scores of all subscales in cases (32.01±13.56 for emotional disturbances, 23.34±9.35 for hirsutism, 12.02±6.6 for infertility, 21.25±10.16 for weight, 16.89±6.71 for menstrual and 20.27±7.25 for acne) were significantly lower than controls (47.53±8.28 for emotional disturbances, 31.81±6.68 for hirsutism, 25.83±4.19 for infertility, 20.23±7.70 for weight, 21.15±7.27 for menstrual and 24.26±6.17 for acne) ($p<0.001$).
Conclusion: According to the results of this study, the average of HRQoL and all the six subscales of MPCOSQ in the case group was significantly lower than in the control group. The psychological implications of PCOS are easily underestimated and have been largely ignored. Clinicians has a pivotal role in recognizing these concerns and implementing therapy to improve quality of life in women with PCOS.
Key words: *Polycystic ovarian syndrom, Quality of life, Infertility.*

P-32
Counseling during pregnancy and women with precedent infertility

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Introduction: Delivery is physiological phenomenon and among the beautiful events in the life of every woman and yet stressful reality. In the present era considering the increasing of caesarean sections, it require using consulting services for informed, correct decisions.
Materials and Methods: In this descriptive-analytical study, 271 pregnant women, completed a researcher-made questionnaire including, demographic characteristics (age and precedent of infertility) and kind and rates of counseling. To analyze the obtained data, chi-square test was used through applying SPSS19 software.
Results: Our results showed that 7 pregnant women (2.6%) had a precedent of infertility while 259 pregnant women had not (95.6%). In total 53 (19.2%) subject did consulting during pregnancy and 213 (80.8%) did not. From these, 6 women with precedent infertility (85.7%) and 47 (14.3%) without, perform counseling during pregnancy. There was a significant relationship between counseling during pregnancy and fertility ($p<0.05$).
Conclusion: The results showed that women with precedent infertility because of the importance of pregnancy and because of the history of information which were obtained during the course of treatment were more acquainted with the role of consultant, and therefor used consultant services mostly during pregnancy. Ferthermore, considering to the high prevalence of elective cesarean, counseling during pregnancy can informe the pregnant women about the advantage of normal delivery, causing the reduction in cesarean section prevalance.
Key words: *Counseling, Precedent infertility, Pregnancy termination.*

P-33
Overweight and obesity of patients: Blind spot in assisted reproductive technology treatment

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Introduction: The effects of lifestyle on health were investigated via several studies. But, the impacts of overweight and obesity on fertility and reproductive system are poorly understood. Overweight and obesity may reduce fertility rate via reducing the IVF success rate in both women and men undergoing treatment via assisted reproductive technology (ART). Improve lifestyle, including physical exercise and weight loss program, may improve fertility and ART outcome.
Materials and Methods: The results of the related epidemiological and randomised control trials (RTCs) sited on Pubmed and ISI database were selected as references.
Results: The results indicated that the patients with a BMI above 30 have about 68% less chance to have a live birth following their first ART cycle compared with women with a BMI less than 30. Also, overweight and obesity of the patients were associated with both lower implantation and more risk for cycle cancellation. A correlation between obesity and the risk of spontaneous abortion (22% increased risk) was observed in obese women compared with normal weight women. The body composition, specific fat mass, was adversely associated with the success of the IVF procedure. Finally, some studies indicate that weight loss is associated with a higher spontaneous pregnancy rate.
Conclusion: Lifestyle behaviours may have a significant impact on pregnancy rates in women with infertility problems. The results support the clinical recommendation of advising overweight and/ or obese

women to lose weight prior to ART. Nevertheless, it seems that the prospective randomized controlled trials are required to prove efficient evidence-based guidelines for weight loss interventions in overweight and/ or obese women before the ART procedure.
Key words: *Lifestyle, Obesity, Fertility, ART.*

P-34
Role of nitric oxide in the developmental process of ovarian follicles in pregnant rats

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Introduction: Nitric Oxide (NO)- as one of the smallest active products, involved in control of a number of physiological processes including cell growth, apoptosis, regulation of blood pressure, defense mechanism and especially in the reproductive process.
Materials and Methods: We used 40 Wistar pregnant rats between 200 and 250 grams in weight and aged eight weeks. Based on observation of vaginal plug, pregnant mice were divided into five groups. The first group was received 2 mg/kg normal saline and the others were received respectively 200 mg/kg L-Arginine, 20 mg/kg L-NAME and a mixture of the same doses of L-Arginine and L-NAME on 3, 4 and 5th gestational days via intraperitoneal. The control group did not received any injection. Ovaries were removed on 18th gestational days, and after fixation and tissue preparation via staining by the routine H&E method, studied by Light microscopy.
Results: Comparing the groups using ANOVA, there was a significant difference in reducing the number of primary follicles and increasing atretic follicles in the L-Arginine group in comparision with the other groups. The histological changes were also observed in the L-Arginine group.
Conclusion: The study results showed that nitric oxide during pregnancy has damaging effects on ovaries and is recommended to be used with caution during pregnancy.
Key words: *Ovarian follicles, Nitric oxide, L-Arginine, L-NAME.*

P-35
Comparison of ovarian stimulation protocols base on AMH level in patients undergoing intracytoplasmic sperm injection

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Introduction: Controlled ovarian hyperstimulation (COH) plays important role in reproductive medicine because selection of appropriate ovarian stimulation strategy can improve assisted reproductive technology outcomes. Antimullerian hormone (AMH) is a predictor of ovarian response, which can help to select the best treatment strategies in women undergoing agonist and antagonist protocols to optimize safety and clinical pregnancy rates.
Materials and Methods: This study is a retrospective study conducted in a private assisted reproductive unite and a total of 243 patients with tubal factor infertility are selected.
Results: In both of GnRH agonist and antagonist protocol, with increasing of AMH level, oocyte and good embryo number is increased. On the other hand in AMH<1.1, > 2.8 ng/ml levels, GnRH agonist leads to higher oocyte and embryo number that is significant. On the contrary, pregnancy rate with increasing of AMH level is not increased and the highest rate of pregnancy is observed in AMH 1.1-2.8 ng/ml levels that it observed with antagonist protocol.
Conclusion: Based on AMH levels we can predict assisted reproductive outcomes. In three range of AMH levels, GnRH agonist protocol can lead to better results. In the women with poor prognosis and low and high levels of AMH, it should focus on improving results with increasing of endometrial receptivity or embryo quality.
Key words: *Antimullerian hormone, Ovarian response, ovarian stimulation protocols.*

P-36
Prenatal exposure to a single dose of testosterone leads to appearance of polycystic ovary syndrome in female rat's offspring in adulthood

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Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women, affecting 8-12% of reproductive-aged women. PCOS is associated with reproductive and metabolic disorders,

including infertility, hyperandrogenism, luteinizing hormone hypersecretion, polycystic ovaries, insulin resistance and dyslipidaemia, with an increased risk of cardiovascular disease and type 2 diabetes in those affected. PCOS is known as a genetic disease. Beside genetic factors, environmental factors may contribute to appearance of this syndrome.

Materials and Methods: Pregnant rats were divided into two groups, experimental and control groups (n=10 in each group). Experimental group were subcutaneously injected with 5 mg free testosterone on gestational day 20 and control rats received only solvent at the same time. The female offspring of these mothers were examined for the functioning of their reproductive system in adulthood.

Results: Levels of testosterone and luteinizing hormone and the luteinizing hormone/follicle-stimulating hormone ratio were increased in prenatally androgenized offspring compared with control rats ($p<0.05$). The numbers of preantral and antral follicles in the ovaries of prenatally androgenized offspring were also increased compared with control rats ($p=0.07$ and $p<0.01$, respectively). The number of corpora lutea was decreased in prenatally androgenized offspring compared with control rats. Cystic follicles were observed in the ovaries of prenatally androgenized offspring.

Conclusion: Prenatal exposure to a single dose of testosterone during the critical period of fetal development leads to appearance of PCOS phenotype in female rats with minimal morphological disorders in their reproductive system in adulthood. Production of a functional rat model that resembles many features of PCOS may contribute to a better understanding of this syndrome.

Key words: *Fetal life, Testosterone, Polycystic ovary syndrome, Rat.*

P-37

Advanced paternal age does not influence the outcomes of ART cycles

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Introduction: The reports in assisted reproductive technology (ART) cycles noted that the fertilization rates were significantly decreased in men over 50 years of age. However, data have shown that there was no adequate substantiation to prove the effects of paternal age on fertility outcomes.

Materials and Methods: In a retrospective study, the outcome of intracytoplasmic sperm injection (ICSI) in ART cycles in two groups of patients was studied. Patients regarding etiology of infertility were divided into two groups: A) male factor infertility (n=47), and B) female factor infertility (n=16). Sperm parameters, ART cycle characteristics and clinical outcomes were assessed between two groups.

Results: There were no significant differences for rates of sperm count and morphology between two groups, but rate of spermatozoa with progressive motility were higher in group B in comparison with A ($p=0.002$). The rates of high quality embryos, pregnancy and live birth showed no significant differences between the groups ($p=0.4$, $p=0.4$, $p=0.2$, respectively).

Conclusion: It appears that the paternal age has no detrimental effect on rates of pregnancy and live birth in ART program.

Key words: *Paternal age, ART, Live birth.*

P-38

Effect of non-polar extract of Phaleria macrocarpa (Mahkota dewa) on serum testosterone level and libido behavior in male rats

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Introduction: Phaleria macrocarpa (PM) has been claimed to overcome male fertility problems related to reduction of testosterone level.

Materials and Methods: Non-polar hexane extract of PM was prepared using soxhlet extraction technique, dried to powder using rotary evaporator and been kept for later use. In total 30 male adult Spraque Dawley rats weight about 250 gr were divided into five groups. For libido study, 30 female rats weight about 250 gr each were used for the purpose of mounting latency and mounting frequency study. The male rats were kept in individual cage while for female rats, there were five rats for one cage. Three groups of rats were given three different concentration of PM non-polar extract [High dose (60 mg/kg), Medium dose (12 mg/kg) and Low dose (6 mg/kg)]. One group was given tween 20 solution (negative control) and another group was given commercial testosterone hormone (positive control) orally for seven weeks. At the end of experiment period each male rat was introduced to one female rat to determine the libido behavior. Blood sample was then collected using cardiac puncture and the testosterone level was determined by radioimmunoassay kit (TESTO-CTK P3093).

Results: Administration of non-polar hexane extract of PM showed no significant changed for the serum testosterone level and mounting latency of the rats. While, there was a significant change ($p<0.05$) in the mounting frequency in different groups; 6, 14, 14, 4 and 7 times for the negative control, low dose, medium dose, high dose, and positive control respectively.

Conclusion: PM non-polar hexane extract did not show a potential value as an alternative way to improve the

sexual strength. However it showed a potential for the improvement of the mounting frequency.
Key words: *Phaleria macrocarpa (PM), Testosterone level, Libido behavior.*

P-39
Effect of vitamin C on sperm parameters and serum malondialdehyde levels in mice fallowing treatment with sodium arsenite

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Introduction: Arsenic as an environmental toxicant is able to exert malformation in male reproductive system by inducing oxidative stress. Ascorbic acid with potent antioxidant property is able to restrict oxidative stress.
Materials and Methods: In this experimental study, 24 NMRI mice were divided into four groups (n=6): control, sodium arsenite (7 mg/kg), ascorbic acid (150 mg/kg) and ascorbic acid + sodium arsenite. Oral treatment was performed for five weeks. At the end, left cauda epididymis was removed and used to analyze sperm viability, morphology, motility and sperm tail length. Serum MDA levels, as lipid peroxidation index, was measured by spectrophotometeric method. Data were analyzed using one way ANOVA and means difference was considered significant at $p<0.05$.
Results: The mean of sperm viability, morphology, motility and sperm tail length significantly decreased in sodium arsenite group compared to control ($p<0.04$). A significant increase in the serum MDA levels was found in sodium arsenite group compared to control ($p<0.05$). In ascorbic acid+ sodium arsenite group, a significantly reversed in adverse effect of sodium arsenite on these parameters was observed when compared with sodium arsenite group ($p<0.02$). In addition sperm viability, motility and sperm tail length was significantly increased in the mice treated with ascorbic acid alone in comparing to the control ones ($p<0.001$). Also, a significant reduction in MDA levels was found in ascorbic acid group compared with control group ($p<0.001$).
Conclusion: Ascorbic acid could compensate the adverse effects of sodium arsenite on viability, morphology, motility, sperm tail length and serum MDA levels in adult mice. Hence, this study suggests that ascorbic acid may improve the male fertility by improving sperm parameters.
Key words: *Sodium arsenite, Ascorbic acid, Viability, Motility, Sperm tail length.*

P-40
The predictors of spiritual growth and interpersonal relations in infertile couples referring to the Infertility Center, Al-zahra Hospital, Tabriz, Iran.

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Introduction: In all cultures, involuntary childlessness is recognized as a crisis that has the potential to threaten the stability of individuals, relationships, and communities. Every society has culturally approved solutions to infertility involving, either alone or together with alterations of social relationships (e.g., divorce or adoption), spiritual intercession (e.g., prayer or pilgrimage to spiritually powerful sites), or medical interventions.
Materials and Methods: This study is a descriptive cross-sectional study on 322 infertile couples referring to the Infertility Center at Al-zahra Hospital in Tabriz. Samples were selected through simple random sampling method. Questionnaires used in the study were demographic data questionnaire and Lifestyle Profile II (HPLP-II) questionnaire with 52 questions. The multivariate linear regression analysis method was used for defining the social-individual predictors of Spiritual Growth and Interpersonal relations. Data were analyzed by SPSS Win/13.
Results: The findings showed that the mean (SD) score of Spiritual Growth and Interpersonal relations in couples was 2.6 (0.5) out of 1-3 grade range. The variable of job, family member, history of contraception, reason of infertility, and education level were the predictors of Spiritual Growth and Interpersonal relations.
Conclusion: According to the results of the study, the mean score of Spiritual Growth and Interpersonal relations in infertile couples is average, therefore we should focus on predictive variables for recovering the infertile couples' Health-promoting behaviors status.
Key words: *Health-promoting behaviors, Spiritual Growth, Interpersonal relations.*

P-41
Comparing in-vitro maturation of oocytes in presence of mature or immature cumulus cells in mice

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Introduction: Cumulus cells (CCs) are somatic cells which are coupled with oocyte during maturation. During process of maturation, the secretions by CCs influence oocyte to maintain the functional competence.
Materials and Methods: Superovulated mice were killed and ampulla was ruptured to release cumulus oocyte complex (COCs) in IVM culture media. COCs were incubated for 30 min before removing oocytes. Then, denuded mature oocytes were removed and remaining mature CCs were collected for culturing germinal vesicles (GVs) for exp II. After dissecting ovaries, denuded GVs were considered as control group

and put in IVM medium. In exp I, collected GVs with intact immature CCs were put in IVM medium and observed for maturation after 24 and 48 hr. In exp II, GVs were cultured with mature CCs and maturation was checked as mentioned for exp I group.

Results: The rate of maturation was (77.36±14.4) in control group. In exp I and exp II, the rate of maturation was (91.32±22.5) and (63.33±7.4) respectively. Difference in maturation rate was significant between two groups of exp I and exp II (p=0.04) at 24 hr. However, maturation rate did not increase after 24 hr. The average of maturation rate in metaphase I (MI) oocytes was as low as about 15% in different groups at 24 hr and did not increase by 48 hr. Degeneration rate was increased from time 0-48 hr in exp II more than this rate in the other two groups.

Conclusion: Presence of mature CC did not improve maturation of GVs comparing to exp I with immature CCs, or control group as conventional IVM.

Key words: *In vitro maturation, Cumulus cells, Mice.*

P-42

Investigating the effect of culture pH, on Chinese hamster ovary (CHO) cells growth and recombinant human chorionic gonadotropin (hCG), expression

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Introduction: The demand for production of recombinant therapeutic proteins by mammalian cell lines, such as Chinese hamster ovary (CHO) cells, continues to grow. Significant achievements in process optimization including development of cell culture strategies for large-scale, cost effective production have been made. One of the key parameters, that has effect on mammalian cell growth, metabolism and productivity, is pH. Human chorionic gonadotropin (hCG), is a member of pituitary glycoprotein hormone family, which has an important role in regulating human reproductive functions and is widely used in assisted reproductive technologies (ART).

Materials and Methods: Equal number of rCHO cells were cultured in four DMEM media with different pH ranging from 6.8-7.7 (6.8, 7, 7.3 and 7.7). The cells were harvested at confluence and viable cell concentration was determined by trypan blue exclusion. The rhCG production was assessed using SDS-PAGE, Bradford and Western blotting techniques. ImageJ software was used for quantitating Western blotting results.

Results: Results demonstrated that maximum viable cell concentration was at pH=7. Bradford assay showed that total protein concentration reached its maximum level at pH=7 and its minimum at pH=6.8. hCG expression was confirmed by SDS-PAGE and western blotting methods. According to ImageJ analysis, maximum hCG expression level was found at pH=7, and minimun expression at pH=7.7 but no significant difference was found at pH=7.7 and pH=6.8. Overall, pH=7 was assessed as optimum pH for culturing rCHO cells.

Conclusion: Several strategies including culture condition optimization, have developed to improve productivity of a recombinant cell line. Findings suggest that optimizing simple parameters such as culture pH, had the potential to increase the viability and productivity of recombinant CHO cells.

Key words: *Chinese hamster ovary (CHO) cell, Optimization, pH, Human chorionic gonadotropin (hCG), Assisted reproductive technology (ART).*

P-43

Noscapine induced cell death in human endometriotic epithelial cells

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Introduction: Endometriosis is the presence of a functional endometrial tissue outside the uterine cavity and defines as cancer like model. Noscapine is a safe cough suppressant which has been introduced as cancer suppressor.

Materials and Methods: Human endometriotic biopsies (n=7) were digested by enzymatic method (collagenase, 2 mg/ml). Epithelial glands were collected by sequential filtration through nylon meshes (70 and 40 µm). The cells were divided to five groups: control and 10, 25, 50 and 100µM concentration of noscapine and cultured for 24, 48 and 72 hr.Viability of cells was assessed by MTT assay; cell morphological analyses with Acridine orange (AO)-ethidium bromide (EB) double staining also cell death was assessed by TUNEL assay.

Results: Viability of endometrial epithelial cells were decreased in 10, 25, 50 and 100 µM noscapine concentration (84.1%, 80.8%, 72.7%, 67.1%) compared to control group (91.0%) in 48 hr respectively. Cell death increased in high concentrations noscapine and were increased TUNEL positive cells in 10, 25, 50 and 100 µm noscapine concentration (9.04%, 13.07%, 15.15% and 17.82%) respectively compared to control group (4.96%) in 48 hr ($p<0.05$).

Conclusion: Noscapineas inducer cell death decreased endometriotic epithelial cells viability and decreased apoptotic index in dose dependent manner. It can suggest for endometriosis treatment.

Key words: *Apoptosis, Epithelial Cell, Endometriosis, Noscapine.*

P-44

The effect of chronic prenatal stress on insulin secretion from Langerhans isolated islets in male offspring rats

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Introduction: A large number of studies have reported associations between prenatal stress and offspring lifetime consequences. Chronic gestational stress alters maternal glucocorticids and subsequently disturbs intrauterine environment which may lead to metabolic disorders in the offspring.
Materials and Methods: In this study the effects of gestational 8 and 20 days foot-shock and psychological stress on body weight, plasma corticosterone, insulin, glucose, concentrations and also insulin secretion from isolated pancreatic islets of rats' offsprings were examined. Stress was induced by Communication Box twice a day (1 h/session) for 8 consecutive days beginning on E8 in 8-day stressed group and for 20 consecutive days beginning on E1 in 20-day stressed group.
Results: The results obtained from this investigation indicate that 8 and 20-day chronic foot-shock stress arises maternal plasma corticosterone concentration. Prenatal stress induces lower birth weight and body weight gain in offspring. Insulin secretion from isolated pancreatic islets of offspring in 8 and 20-day foot-shock stress groups in presence of 8.3 and 16.7 mM glucose increased as compared to the control and psychological groups. Furthermore, prenatal stressed offspring had significant elevation in plasma glucose concentration without marked alteration in plasma insulin and corticosterone concentrations.
Conclusion: These data suggest that prenatal stress could result in impaired glucose metabolism in the offspring which is independent of timing of the stress exposure.
Key words: *Prenatal stress, Corticosterone, Insulin, Glucose, Langerhans isolated islets.*

P-45
In vitro culture of vitrified mouse ovarian tissue derived preantral follicles in two and three dimensional systems

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Introduction: Cryopreservation is the best choice to preserve fertility in patients exposed to premature ovarian failure. Designing an appropriate system for *in vitro* follicle culture that resembles *in vivo* conditions is really valuable and could improve the usage of cryopreserved tissue.
Materials and Methods: Ovaries of 12-day-old female NMRI mice were exposed to EG and DMSO combination (7.5% and 15%) in two steps for 15 and 30 minutes respectively, then cryopreserved by needle immersed method (NIV). Afterwards, middle sized preantral follicles (110-130 µm) were mechanically isolated and were distributed into two different groups: two dimensional culture system (2D) and three dimensional culture system (3D; alginate encapsulation). Both groups were cultured for 12 days in α-MEM that supplemented with 10 mIU FSH, 1% ITS, 50 ng/ml activin A and 5% FBS. Finally, follicular morphology, survival, growth rate and also quantitative expression of oocyte maturation genes (*Gdf9*, *Bmp15* and *Bmp6*) were studied on the first and last days of culture.
Results: At the end of culture period, although follicular morphology in 3D culture system was better preserved as compared to the 2D one, survival rate of cultured preantral follicles was significantly ($p<0.05$) lower than in 2D group. Expression analysis of oocyte maturation genes indicated a reduction process in both groups during 12 days of culture. In the last day of culture period, genes expression level in 3D culture system was significantly lower than 2D group.
Conclusion: Although the better survival rate was seen in 2D culture system, morphologic characteristics including antrum formation and follicle development was extremely better preserved in 3D culture system. Overall, as three dimensional culture system mimic in vivo conditions, so could be used as a more appropriate system for follicle culture.
Key words: *Ovarian tissue, Preantral follicle, Two dimensional culture system, Three dimensional culture system, Vitrification.*

P-46
Routine use of EmbryoGlue® as embryo transfer medium does not improve the ART outcomes

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Introduction: The assisted reproductive technology (ART) success rates have been significantly improved over last decades; however, embryo implantation still remains a major limiting factor. The composition of an embryo transfer (ET) medium is important for interaction between embryo and endometrium at the time of implantation. Some modifications to the embryo culture media are developed to mimic the in vivo conditions. One of the examples for that is supplementation of ET media with hyaluronan (HA, hyaluronic acid), a major glycosaminoglycan in uterine fluid, which assumed to improve the process of implantation.
Materials and Methods: A cohort of total 229 patients was retrospectively enrolled for the present study. They were subjected for ET on day 2 either in EmbryoGlue® (n=117) as study group or in conventional ET medium with low concentration of HA as control group (n=112).
Results: Patients in the both groups, in regards to the mean level of day 3 FSH, the etiology of infertility, the history of implantation failure and the rate of good quality embryos showed similar characteristics. There were no significant differences between two groups in terms of clinical and ongoing pregnancies, implantation, delivery and live birth rates. In spite of a decreased abortion and increased multiple pregnancy rates in the study group compared to the control group (15.8 vs. 19% and 20.6 vs. 15.6 respectively), the differences were not statistically significant.
Conclusion: Routine use of EmbryoGlue® as a HA enriched ET medium for cleavage stage embryos does not have advantage to the conventional one for infertile patients undergoing ART.
Key words: *Hyaluronan, EmbryoGlue®, Embryo transfer, Pregnancy outcome.*

P-47

The relation between vitamin C in follicular fluid with the morphology of oocyte and the quality of embryo in IVF patient

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Introduction: Oxidative stress and the inappropriate effect of reactive oxygen in the body or laboratory circumstances can decrease the quality of embryo and sexual cells. Ascorbic acid is a natural antioxidant which has a protective effect in the body.
Materials and Methods: This is a cross-sectional analytical study which was conducted on 50 women with IVF referred to Al Zahra Hospital, Rasht, Iran. 20-45 years old women with infertility caused by tubal dysfunction, ovarian factors or male factors were included. Patients underwent same protocol for stimulating; the ovulation and injection of 10,000 units of hCG. After 36 hr, follicles were suctioned and matured oocytes were separated for fertilization. Vitamin C was assessed biochemically and the morphology of oocyte and the quality of embryo were evaluated by by inverted optical microscope.
Results: Among patients 583 oocytes and 275 embryos were assessed. There was no significant relation between, age, BMI and duration of infertility with vitamin C ($p>0.05$). Also there was no significant relation between vitamin C, the maturity of oocyte and the quality of embryo ($p>0.05$). But MII oocytes were more in patients with vitamin C less than 1 in comparison with ≥1 ($p=0.038$). Also the mean 2PN embryos in 0-0.05 level of vitamin C was higher than other groups but no significant relation was noted ($p=0.719$).
Conclusion: According to our results vitamin C at special level can improve the morphology of oocytes and the quality of embryos.
Key words: *Infertility, Follicular fluid, IVF, Vitamin C, Oocyte, Embryo, Antioxidants.*

P-48

Polscope analysis of meiotic spindle and zona pellucida birefringence of metaphase II oocytes in polycystic ovarian syndrome patients

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Introduction: Polycystic ovarian syndrome (PCOS) is one of the most common causes of infertility in women of reproductive age. Currently, one of the best therapeutic options for PCOS patients is ICSI. Moreover, IVM can be a useful technique for women with PCOS who are at the risk of ovarian hyperstimulation syndrome (OHSS). On the other hand, the oocyte quality can be a determining factor for outcome of ICSI cycles.
Materials and Methods: This prospective study included immature oocytes (30 GV and 5 MI) undergoing IVM, and MII oocytes obtained from PCOS patients (29.64±5.31 years) in ICSI program. Using a PolScope, the presence of MS and ZP birefringence was assessed in both in vivo-matured (n=32) and matured oocytes after IVM (n=24). Oocytes were classified as high birefringent (HB) ZP and low birefringent (LB) ZP. Furthermore, the rates of fertilization and embryo development were evaluated.

Results: The maturation rate was 68.5% after IVM. Analysis revealed that the percentage of a HB ZP was significantly higher in the IVM oocytes than in vivo-matured ones (58.3% vs. 31.2%, p=0.04). There was insignificant relationship between spindle detection and either in vivo-maturation or IVM oocytes (p=0.53). Likewise, there were similar outcomes for the rates of fertilization and embryo development after ICSI between two groups (p=0.80 and p=0.13, respectively).
Conclusion: Clinical IVM is a safe technology for the maturation and maintenance of oocytes integrity. Furthermore, the use of non-invasive PolScope is recommended for the detection of healthy oocytes in ICSI.
Key words: *PCOS, IVM, PolScope, ZP birefringence, Meiotic spindle.*

P-49
History and application of public bank of umbilical cord blood

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Introduction: Umbilical cord blood is rich of hematopoietic stem cells. Today, umbilical cord blood constitutes about 20% of stem cells transplant. 75% of umbilical cord blood banks in the world are public and the remaining are private (trade). 27 years after the first successful umbilical cord blood transplant, more than 555,000 umbilical cord blood units have been made available around the world after performing qualitative study in public bank of umbilical cord blood in order to treat patients who need hematopoietic stem cells transplant. The first program of public umbilical cord blood bank was implemented by the New York in 1991 and in Asia in 1999 in Japan. In Iran in late 2008, the Stem Cells Association has started its activity by receiving financial supports. In this type of storing, umbilical cord blood does not only belong only to the family and public organizations are responsible for paying related costs. Cells in this banking are kept safe and number of their alive cells remains unchanged during the freezing process. Samples saved in bank in terms of health indices such as viral and microbial contaminations are evaluated by colony measurement method. With regard to high prevalence of blood malignancies and thalassemia in Iran, it has been very important to establish public bank of umbilical cord blood and support it spiritually and materially.
Materials and Methods: Subjects of this review study are derived from articles and sites related to collecting, maintain and transplanting umbilical cord blood.
Results: Development of umbilical cord blood banks to develop transplantation centers and treat patients is of unavoidable necessities in the society health system. In societies that the government has ability to establish public bank and supports this bank, development of public banks has preference to development of private banks.
Conclusion: In year 2011, research and development department established public bank of umbilical cord blood in order to scientific and practical promoting and proceeded to amniotic membrane, standardization of tissue adhesive, production and standardization of amniotic extract and platelet extract by establishing bank. Collecting this bio garbage and transforming it to biologic bands, eye drop to improve cornea wounds and mixing hydrogen with amniotic membrane extract to treat wounds for therapeutic consumptions is economical and is effective to decrease therapeutic costs. Also fibrin glue is produced only via umbilical cord blood. Fibrin glue as cell carrier and tissue adhesive is widely used in surgery and cell therapy. Transplant of stem cells of umbilical cord has opened bright and promising horizons for medical society to treat patients with different chronic and acute disorders. So, saving umbilical cord blood finds high importance and its necessity feels further.
Key words: *Umbilical cord, Blood bank*

P-50
Protective effect of alcoholic extract of Garden cress (*Lepidium sativum*) seeds on the histopathological changes of epididymis and fertility in diabetic rats

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Introduction: Diabetes mellitus (DM) is a frequent and serious metabolic illness all over the world and plants have been a desirable source of medicine recently. Diabetes has unpleasant effect on male reproductive system and it may lead to male infertility. It causes erectile dysfunction and reduces ejaculate volume by affecting the health of small blood vessels and the small nerves that control ejaculation and also decrease libido by decreasing testosterone levels. Current study evaluated the possible protective efficiency of Garden cress (*Lepidium sativum*) seed extract on fasting blood sugar (FBS) and then assessed epididymal histopathologic changes in streptozotocine (STZ)-induced diabetic rats.
Materials and Methods: 50 male adult Wistar rats were randomly categorized into five groups (each 10 rats). Groups 1 were control placebo group receiving only 0.1 ml normal saline via gastric *gavages*, Group 2 as control diabetic rats received an intraperitoneally injection of STZ 60 mg/kg body weight. Rats with FBS >250 mg/dl were considered as diabetic. Group 3 were diabetic rats receiving insulin in dose 3U/100 g body

weight and Groups 4 and 5 were diabetic rats that received 0.1 cc of 200 and 400 mg/kg, ethanol extract of *Lepidium sativum* seed by gavages daily. One day after the last gavages, rats were anesthetized by chloroform. Epididymis duct was removed from abdomen and weighed with a digital scale. Afterwards, samples were putted in Bouin's solution for histological measurement.

Results: Administration of 200 and 400 mg/ml doses of *Lepidium sativum* seed extract increased epithelium height and decreased interstitial volume density and fibro muscular thickness significantly. Also, volume density of epithelium, fibro muscular, lumen and interstitial were decreased significantly. Tubular and lumen diameter did not change significantly in different groups.

Conclusion: It seems *Lepidium sativum* seed extract has benefit as a supplementary protective agent against bad effects of diabetes on reproductive system in diabetic male.

Key words: *Diabetes, Lepidium sativum seed extract, Epididymis, Streptozotocine, Insulin.*

P-51

New insights into the effect of aflatoxin on infertility of animal models

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Introduction: Aflatoxins are produced via Aspergillus flavus and Aspergillus parasiticus, species of fungi. Toxic and carcinogenic properties of Aflatoxin are determined. After entering the body, Aflatoxins may be metabolized by liver to a reactive epoxide intermediate or hydroxylated to become the less harmful. Nowadays, infertility properties of Aflotoxine have been known in studies.

Materials and Methods: Literature review was conducted using the following databases: SciELO, Lilacs, Medline, PubMed, Scopus and science direct from 2000-2014. The key-words used were Aflatoxin, infertility, fertility, animal, human.

Results: When Aflatoxin level increased in diet of male goats, glucose and total protein concentration were decreased in the testes and testosterone level was significantly reduced. The study suggests that exposure of male goats to dietary Aflatoxin will reduced testicular biochemical and testosterone with resultant depression in sperm storage capability and daily sperm production in the animals. Chronic Aflatoxin exposure in animals can result in impaired reproductive efficiency. One study showed that Aflatoxin decreased fertility, abortion, lowered birth weights and caused disturbances in hormonal metabolism in sheep. Another study showed that the fertility of treated mice with Aflatoxin at a daily dose of 50 µg/kg body weight was reduced drastically. Sperm concentration in the epididymis and sperm motility decreased whereas sperm abnormalities increased.

Conclusion: The results of studies showed that Aflatoxin can cause infertility in animal models, but these results need to be evaluated by more clinical trials.

Key words: *Aflatoxin, Infertility, Animal.*

P-52

ART cycles outcome in donor oocyte recipient couples: A case-control study

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Introduction: Oocyte donation is an acceptable alteration in women infertility treatment for different indications. Several factors including the age of the oocyte donor and recipient, oocyte and embryo quality and characteristics of endometrium can influence on ART outcome.

Materials and Methods: Outcome of 202 cycles, consist of 113 DO cycles and 89 AO cycles, were compared. Both DO and AO groups were divided to two subgroups according to fresh and frozen embryo transfer. First DO and AO groups included the fresh embryo transfer (fDO, fAO) and another DO and AO groups included the frozen embryo transfer (fzDO, fzAO). Comparison was performed in regard to chemical pregnancy, clinical pregnancy, implantation rate, live birth and abortion rate.

Results: DO (mean=28.29 years) and AO (mean=29 years) groups were significantly matched by age. No significant differences were observed between fDO and fAO groups and also between fzDO and fzAO groups with regard to chemical pregnancy, clinical pregnancy, implantation rate, live birth and abortion rate. But, there was a significant increase in oocyte number, MII oocyte number and embryo number in DO compared with AO groups ($p<0.001$).

Conclusion: Despite of increasing in total number of oocyte, MII oocyte and embryo, our findings showed that oocyte donation program has no impact on ART pregnancy outcome.

Key words: *Oocyte donation, ART outcome, Women infertility.*

P-53

Dose stress always has a negative effect on sperm cell?

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Introduction: Cryopreservation of semen requires optimized condition to minimize the harmful effects of various stresses. Oxidative stressors are known to be major mediators of cell damages during cryopreservation. An important perspective is that free radicals are not exclusively beneficial or exclusively detrimental. Rather, they need to be maintained at appropriate levels to ensure physiological function, while preventing pathological damage. Recent studies showed that placing the cells in a controlled stress conditions could affect cell survival in later stages.
Materials and Methods: This article discusses some of the new approaches employed for improve spermatozoa before and after freezing-thawing. A review of recent bibliography was carried out in PubMed, Google scholar and SID by the use of relevant keywords.
Results: Osmotic, oxidative, hydrostatic and mechanical stressors before freezing induce expression a series of heat shock proteins (HSPs), decrease apoptotic sperm amount and increase live sperm during treatment and high mitochondrial activity observed in treatments. Cell's reaction to environmental stresses is depended on domain, concentration and acts time of this reactions.
Conclusion: Although cellular and sub-cellular mechanisms, supposedly contributing to these processes, require further research, the new principle may outline a completely new strategy in mammalian embryology, as well as cryopreservation of other cells and tissues with remarkable theoretical and practical consequences.
Key words: *Cryopreservation, Sperm, Stress, Sublethal.*

P-54
The effect of KIT ligand (KITL) and leukemia inhibitory factor (LIF) on maturation and apoptosis of follicles after vitrification of mouse ovary and three dimensional culture

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Introduction: In vitro ovarian follicle culture techniques not only provide a model for research into the mechanism of folliculogenesis but also, in combination with cryopreservation of ovarian tissue, it may have clinical applications in preserving of fertility.
Materials and Methods: This experimental study is carrying out on 7-days-old female mice (NMRI). In the first step, the ovaries were vitrified with a solution containing ethylene glycol and then their morphology, ultrastructure and apoptosis were compared with non-vitrified ovaries. In the second step, the non-vitrified and vitrified ovaries were cultured in base medium α-MEM supplemented with KL and leukemia inhibitory factor (LIF) for 7 days then their morphology, area, apoptosis, hormone assay were analyzed. In third step, mechanically isolated preantral follicles were cultured in two dimensional and three dimensional systems in α-MEM supplemented with 5% FBS, 100 mlU/ml, 1% ITS, 10 ng/ml rEGF and KL for 12 days then the ovulation were induced. At the end of culture survival and maturation rate, apoptosis and hormones were assayed. In the fourth step, the level of reactive oxygen species (ROS) and ATP, distribution of mitochondria and fertilization rate in MII oocyte were assessed.
Results: The results of first step showed that the morphology, ultrastructure and apoptosis of vitrified ovaries were similar to non-vitrified ovaries. The results of second step showed that the percentage of preantral follicles, ovarian area, production of hormone in the non-vitrified ovaries cultured in medium supplemented with KL were significantly higher and the level of caspase 3/7 was lower in comparision to other groups ($p<0.001$). The results of next step showed that the survival, maturation rates and production of hormone of follicles in KL supplemented in three dimensional system groups were higher than other groups. The level of caspase-3/7 activity was lower in three dimensional culture systems ($p<0.05$). The results of fourth step showed that the level of ROS and ATP in MII from in vitro culture of follicle had significant different with control in vivo group and the rate of embryo development were significantly higher in three dimensional culture system.
Conclusion: Our results demonstrated that the culture of ovarian tissue and three dimensional culture of ovarian follicle in combination of KL improved the growth and development of ovarian primordial follicle.
Key words: *Vitrification, In vitro maturation, Apoptosis, Mitochondria, KIT Ligand (KITL).*

P-55
Evaluating the levels of expression of TLR 9 in women with endometriosis and its comparison with normal endometria

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Introduction: Endometriosis is a benign condition which endometrial glands and stroma appear outside the uterine cavity which presents by pelvic pain and infertility. Endometriosis is associated with changes in cellular and humeral immunity and impaired immune response, leading to inefficient removal of debris after a menstrual cycle. Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. Based on recent studies TLRs are increased in endometriosis and initiate immune responses.
Materials and Methods: This case-control study assessed and compared expression of Toll like Receptor 9 in the endometrium of three type tissues: 1) Eutopic endometria of women with endometriosis 2) Ectopic

endometria women with endometriosis 3) Endometrium of women without endometriosis. Eutopic and ectopic endometrial samples were taken from 10 patients with endometriosis (Case Group). Also endometrial samples were taken from 10 patients without endometriosis or infertility history whom operated for other gynecological cause. The level of TLR9 gene expression in ectopic samples, eutopic and controls were evaluated by Real Time-PCR. Patients were chosen randomly from Arash Hospital. Data was analyzed by SPSS.

Results: Real Time-PCR showed that TLR9 expressed in all three groups of eutopic, ectopic and control. According to statistical analysis, level of TLR9 was higher in ectopic group, but not significantly (p=0.13). There was no significant difference between eutopic and control groups.

Conclusion: Considering the role of TLR9 in the innate immune system, such as pathogen detection and set up a cascade of inflammatory response associated with cell proliferation and inhibition of apoptosis, this study approves the association between TLR9 and endometriosis. Because of lack of information about this new issue, we suggest further researches and more studies.

Key words: *TLR9, Endometriosis, Inniate immune system.*

P-56

Congenital uterine malformations: A widespread phenomenon among infertile women with recurrent miscarriages and IVF failure

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Introduction: Uterine malformations are a various group of congenital uterine disorders found in women with recurrent abortions or IVF failure.

Materials and Methods: A narrative review was performed within articles published at "PubMed", "Elsevier", "EBSCO", original text books and etc. to reach the aim. Several unique high-quality images were provided in this article, using the archive of infertile patients referred to imaging department of Royan Institute.

Results: Congenital uterine anomalies are originated from development defects of mullerian ducts during fetal growth. They are also associated with higher incidences of infertility, preterm birth, intra uterine fetal death and etc. However, manifestations and severity of the obstetric/ gynecologic complications and treatment procedures vary depending on the type of anomaly. Thus, accurate diagnosis of uterine malformations and differentiation between various types of them, have a vital role in decision about treatment procedures and management of these patients. Several imaging modalities are used to investigate women suspected to have uterine anomalies. In this article, we described about various types of uterine malformations and imaging evaluation of them prior to IVF treatment cycles.

Conclusion: Congenital anomalies of the uterus are a major cause of recurrent miscarriage and pregnancy failure. Several imaging methods help midwives and obstetricians evaluate this group of infertile women to detect suspected uterine anomalies.

Key words: *Congenital uterine malformation, Infertility, Recurrent miscarriages.*

P-57

Study on the impacts of anti-histamine dimenhydrinate on testicular tissues and sperm production of male mature rat

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Introduction: In this study we did a research about the effects of AHDH on testicular tissues and sperm production of rats. Dimenhydrinate (DH) is used as anti-nausea as well as an anti-gastric reflux in cases of nausea caused during travelling. It is easily taken orally and it will be excreted by the kidneys within 24 hr.

Materials and Methods: This survey is conducted in laboratory on experiment basis among 30 mature male rats categorized in 3 different groups; two experimental groups and one control group. The control group was watered normally for 60 days and the first experimental group was watered with 200 mg DH blended in their water equal to one kg of their body weight for 60 days. In the end, after the rats were anesthetized, testicles were removed, sectioned and painted with Hematoxilin and Eozine and were investigated.

Results: The obtained results have revealed that in the experimental groups, testicles weight decreased substantially in comparison to those of the control group ($p<0.001$). The reduction of the number of sperm cells within the experimental group, in comparison to those of the control group was significant ($p<0.001$). The reduction of the Leydig cell was very substantial in the second experimental group ($p<0.001$). The difference of the Sertoli cells was not noticeable in neither of the experimental groups.

Conclusion: DH has impact on testicular tissues and weakens their mechanism.

Key words: *Dimenhidranate, Testis.*

P-58
Investigating association of G103T polymorphism of Coagulation factor XIII and recurrent pregnancy loss

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Introduction: Recurrent miscarriage (RM) occurs in 1-3% of couples that attempting to bear children. Thrombophilia is one of the suspected cause of recurrent miscarriage. At the end of coagulation cascade there is Factor XIII that makes blot stable. The polymorphism G103T of factor XIII gene is the most common polymorphism that affects FXIII's activity.
Materials and Methods: The study groups consisted 50 patients with two or more consecutive miscarriage. The control group included 50 women with at least two successful delivery and no history of pregnancy loss. By using PCR-RFLP, DNA from both groups analyzed for carrying mutation of FXIII.
Results: 4% in the case group were homozygote (TT) for 34 Leu mutation whereas no homozygote (TT) was found in control group ($p<0.05$). 28% patients in the case group and 26% women in the control group were found to be heterozygote for G103T polymorphism ($p>0.05$). No significantly differs was observed between patients with RPL and healthy women for G103T mutation.
Conclusion: No statistically differs between case group and control group was observed.
Key words: *Recurrent miscarriage, Thrombophilia, F XIII.*

P-59
Effect of pumpkin seed and ginger extracts on adult rat's sperm characteristics, biochemical parameters and epididymal histology treated with cyclophosphamide

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Introduction: Infertility is one of the problems of human society and 10-15% of couples have experienced some forms of infertility problems. Reproductive toxicity is one of cyclophosphamide (CP) side effects in cancer treatment.
Materials and Methods: Male adult Wistar rats were randomly divided into six groups. Group 1 as control received an isotonic saline solution injection intraperitoneally (IP). Group 2 was injected intraperitoneally with a single dose of CP (100 mg/kg) once. Group 3 and 4 received CP plus 300 and 600 mg/kg combined pumpkin seed and Zingiber officinale extract (50:50). Group 5 and 6 received only 300 and 600 mg/kg combined pumpkin seed and Zingiber officinale extract. Six weeks after treatment, sperm characteristics, histopathological changes and biochemical parameters were assessed.
Results: Results showed that in CP treated rat's sperm characteristics were diminished significantly. Biochemical analysis showed that the administration of combined extracts could increase the Total Antioxidant Capacity (TAC) level significantly in groups 3, 4, 5 and 6. Also, in these groups, the sperm viability, motility, count, normal sperm morphology, epididymal epithelium and fibromascular thickness were improved compared to control and CP groups. Interestingly, the mixed extract could improve histopathological changes such as vacuolization, disorganization and separation of epididymal tissue in CP treated rats as well.
Conclusion: Overall our findings indicated that the combined extracts might be used as a protective agent against CP-induced reproductive toxicity.
Key words: *Pumpkin seed extract, Zingiber officinale extract, Cyclophosphamide, Total antioxidant capacity (TAC), Sperm parameters, Rat epididymis.*

P-60
Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: A randomized clinical trial

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Introduction: Frozen- thawed embryo transfer is an essential part of ART treatment and outcomes of this procedure are associated with several clinical factors. Several studies have showed an increase level of IVF outcomes in women with sufficient vitamin D. whether treatment of vitamin D insufficiency can improve pregnancy rates in frozen-thawed embryo transfer cycles.
Materials and Methods: This is an interventional, randomized clinical trial. Serum 25-(OH) vitamin D level of 128 women who had undergone IVF/ ICSI with cryopreservation of embryos was checked. One hundred fourteen infertile women with insufficient serum vitamin D (less than 30 ng/ml) were included in the study. Fifty seven women were treated with supplementary vitamin D, 50000 IU weekly, for 6-8 weeks and 57 women were received no

supplementation. 106 women completed frozen thawed embryo transfer cycles and included in the final analysis. Primary and secondary outcomes were chemical and clinical pregnancy respectively.
Results: Our study did not show any significant difference between vitamin D insufficient and treated women in term of chemical (29.40% vs. 29.10% respectively, p=1.00) or clinical (25.50% vs. 21.80% respectively, p=0.81) pregnancy rates.
Conclusion: Vitamin D insufficiency treatment is not associated with higher pregnancy rate in frozen-thawed embryo transfer cycles.
Key words: *Vitamin D, Embryo transfer, Pregnancy rate.*

P-61
Association of rs10954213 polymorphism of *IRF-5* gene with idiopathic recurrent miscarriage

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Introduction: Interferon regulatory factor (IRF) 5 is a transcription factor that can induce transcription of IFN-α mRNA. Recent studies have shown that IRF-5 promotes the proliferation of T cells and the activation of TH1 and TH17 cells but does not induce TH2 or Treg differentiation. TH17 cells produce inflammatory cytokine IL-17 which plays an important role in the induction of inflammation via neutrophil infiltration and stimulation of inflammatory cytokines. There are many reports that inflammatory processes play a major role in recurrent miscarriage in a manner that an uncontrolled and persistent inflammatory response during pregnancy can harm placental growth. We analyzed the rs10954213 polymorphism of *IRF-5* gene in normal pregnancy and recurrent miscarriage patients in order to discover a possible mechanism for the genetic control of immune regulation in patients with recurrent miscarriage.
Materials and Methods: Genomic DNA from 100 recurrent miscarriage (RM) patients and 100 normal fertile control individuals using the routine salting out method were isolated. DNA fragments were then analyzed by real time PCR with SYBR Premix Ex Taq II master mix. Statistical analysis used SPSS 19 software. *IRF-5* allele frequencies and genotypes in RM women and the fertile control group were compared using a Chi-square test.
Results: Our results so far indicate that there is a detectable relationship between rs10954213 polymorphism of *IRF-5* and susceptibility to recurrent miscarriage.
Conclusion: *IRF-5* gene plays a possible role in the induction of inflammation and therefore leads to recurrent miscarriage. However, additional studies are needed in this regard.
Key words: *Rs10954213, IRF-5, Recurrent miscarriage.*

P-62
Role of 45,X mosaicism in couples with fertility problems

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Introduction: Infertility is typically defined as the biological inability of a person to contribute to conception after one year of attempting to have a child. Genetic factors such as chromosomal abnormalities are one of the major causes of infertility and spontaneous abortions. The objective of this study was to establish chromosome X abnormality rate among women referred for infertility problems to Sarem Women's Hospital in Tehran between October 2007 and January 2015.
Material and methods: A total number of 1764 women with the age range of 12-60 years were referred for chromosomal investigation. Referral reasons included recurrent abortions, primary infertility, secondary infertility, Turner syndrome, premature ovarian failure, amenorrhea and ART failures. Cytogenetic analysis was performed on cultured peripheral blood lymphocytes stimulated with phytohaemagglutinin M, using standard techniques. Karyotype was done in all patients using high resolution GTG banding technique. For each patient, at least 15 chromosome spreads were examined by light microscopy, and extended to 100 in the case of mosaicism.
Results: Cytogenetic investigation was performed on 1764 patients. The overall chromosome X abnormality rate in infertile and subfertile women was 4% (74 out of 1764). The cytogenetic result for these patients with fertility problems is as follows: 74 patients had a numerical chromosome abnormality and 65 (85%) of them had mosaicism of 45,X with other different cell lines. The age range of women with 45,X mosaicism was 12-53 years old.
Conclusion: Accurate genetic diagnosis is the most important prerequisite for genetic counseling in patients with fertility problems. Finding of chromosome X numerical abnormality are of great value in better management of the patients. However, the attribution of chromosome X instability due to age should be considered in Genetic counseling of these couples.
Key words: *Fertility problems, Chromosome X abnormality, 45,X cell line.*

P-63
Effects of mitochondrial gene deletions on human sperm motility

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Introduction: Asthenozoospermia is an important cause of male infertility. It has been primarily characterized by reduced sperm motility owing to a variety of factors, including ultra-structural abnormalities, abnormal semen liquefaction, anti-sperm antibodies, varicocele and endocrine abnormality, etc. The mitochondrion is the major energy provider for sperm motility. Mitochondrial DNA contains several genes encoding for proteins that play an important role in oxidative phosphorylation and in ATP production. Mutations in sperm mtDNA result in either functionless or malfunctioning proteins, subsequently affecting sperm motility leading to asthenozoospermia.
Materials and Methods: To detect 4977 bp deletion in spermatozoa mtDNA, semen samples of 42 asthenozoospermic infertiles and 50 controls from northern Iran were collected. After extraction of spermatozoa total DNA, Gap-PCR was performed.
Results: 4977 bp deletion was observed in 73.80% of patients with asthenozoospermia, compared with 36% in controls (OR=5.0101, 95% CI: 2.0408-12.2998, p=0.0004).
Conclusion: Large-scale mtDNA deletions in spermatozoa may induce bioenergetic disorders and cause subfertility or infertility in men. It is concluded that there is a strong association between sperm mtDNA 4977 bp deletion and asthenozoospermia-induced infertility in the population examined. Nevertheless, to validate our results broader research may be needed.
Key words: *Asthenozoospermia, mtDNA deletion, Male infertility, Motility.*

P-64
Effects of Nano TiO2 on chromatin, apoptosis and parameters of sperm in mice

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Introduction: Titanium dioxide nanoparticles (TiO_2 NPs) are manufactured worldwide and used in a broad range of applications. In mammals, there are only limited reports regarding the effect of TiO_2 NPs on male reproductive system. It has been demonstrated that TiO_2 NPs taken up by mouse Leydig cells reduced the viability and proliferation of these cells. Also it is improved that titanium compounds can disrupt the mouse blood-testis barrier. In this study we decide to evaluate the effects of nano-TiO_2 on chromatin, apoptosis and sperm parameters of male mice.
Materials and Methods: 35 NMRI male mice were classed in five groups: control, sham and three treated groups. Low, medium and high treated groups were gavaged 2.5, 5 and 10 mg/kg doses of TiO_2 respectively for thirty five days. Sham group only received saline instead. The mice were weighted before and after administration. Sperm count, motility, morphology and viability were analyzed accordingly. Sperm DNA integrity and apoptosis were assessed using acridine orange, aniline blue, toluidine blue, CMA3 and TUNEL assay.
Results: Non-significant decrease in body weight gain was observed. Sperm analysis showed no count, motility and viability changes but morphological changes were significant. Most of the morphological abnormalities were observed in sperm neck and tail. Tail morphological changes in treated groups were significant in comparison to non-treated ones ($p<0.001$). Compared to non-treated groups, sperm neck changes in medium and high treated groups were significant but not in low treated group ($p<0.001$). Results of acridine orange, aniline blue, toluidine blue and CMA3 were non-significant in all groups. Also increasing apoptotic cells in treated groups were discovered by TUNEL assay but there were non-significant statistically.
Conclusion: TiO_2 significantly may increase sperms with abnormal morphology especially in tail and neck part. Also apoptosis boosting occurred but the exact mechanism is still controversial.
Key words: *Nano-TiO_2, Sperm parameters, Apoptosis.*

P-65
Protective role of vitamin C on hazardous effects of acrylamide in rat offspring

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Introduction: Acrylamide (ACR) is a substance chemical used in industrial and laboratory procedures. Acrylamide according to the method of cooking foods are increasingly used and its adverse effects on multiple organ systems have been described sporadically in the literature. The purpose of this study was to evaluate the effects of ACR during pregnancy and lactation on the development and changes in cortical layer of the cerebellum and cerebellar Purkinje cells in fetal and neonatal rat by using histological and morphometric and stereological technique.
Materials and Methods: In this study 20 adult female Wistar rats weighing 180 gr and aged two months were used. Animals were randomly divided into four groups. Female pregnant rats were orally administered 10 mg/kg ACR and/or 200 mg/kg vitamin C (vit C). Pregnant rats

were sacrificed on the 15th day of gestation and mother's weight was measured. After that, their fetuses were taken out and were evaluated for fetus number, weight, crown-rump length (CRL) and cerebellar development. To study the neonatal period, 6 infants at day 21 were randomly selected and placed under deep anesthesia and transcardial perfusion. The cerebellum was taken out and fixed and cerebellum changes were evaluated by crysel violet and Hematoxylin-Eosin staining method. The cerebellar cortex layers volume were investigated by Cavalieri's principle method. Data were analyzed by SPSS software and by ANOVA and LSD Test. $P<0.05$ considered as statistically significant.

Results: The results showed that ACR decreased fetal weight and CRL, but this reduction in weight and volume of the cerebellum $p<0.001$ and the number of embryos with $p<0.05$ was significant. Histological and stereological examinations revealed that the cerebellar volume was decreased in ACR and ACR+vitC group vs control ($p>0.001$). While in vitamin C group the cerebellar volume was inversed ($p>0.05$). ACR in newborn decreases body weight, brain weight, thickness of cerebellum with $p>0.001$. The extent of this reduction in the weight of the cerebellum was significant with $p<0.05$.

Conclusion: ACR exhibits a harmful effect on the development of the cerebellar cortical layers, which may be prevented by administration of vit C as an anti oxidant.

Key words: *Acrylamide, Vitamin C, Cerebellum, Development, Rat.*

P-66
Robust stem cell isolation from human dental pulp

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Introduction: Dental pulp stem cells (DPSCs) are a population of clonogenic and highly proliferative cells derived from enzymatically digested Dental pulp tissue. Providing scaling-up of stem cells at early passages is of importance for regenerative medicine purposes. Typically, two protocols are employed to isolate stem cells from human dental pulp: tissue explants culture and enzyme digestion of pulp tissue and culture of released cells.

Materials and Methods: Dental pulp was extracted from third molars of 60 healthy subjects. In the first method pulp was digested with 1 mg/mL collagenase/ dispase (Roche) for 30 min and released cells obtained using a 70-µm cell strainer for culture, in the second method intact pieces of pulp were cultured and in the third method digested pulp pieces were immobilized and cultured. The cells and tissues maintained in alpha-MEM supplemented with 20% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 ng/mL amphotericin B and incubated in humidified incubator with 5% CO_2 at 37°C. In each group cells and colonies were counted and compared.

Results: Results showed that treating pulp segments with enzymes and culturing them (combinatory method) increased the efficiency of cell isolation up to 60% significantly in 3-4 days of culture compared with other methods which this value was <20% in 10-15 days.

Conclusion: According to the small size of pulp tissue and its low stem cell contents, acquiring substantial quantities of cells in primary culture will facilitate the in vitro expansion and providing adequate production of the stem cells at early passages with minimum risk of losing their 'stemness' and aberrant genetic changes for use in research, tissue engineering and regenerative medicine. Optimized method increase efficiency of cell isolation and provides significant quantities of stem cells in primary culture more than other methods.

Key words: *DPSCs, Cell isolation, Cell culture.*

P-67
Evaluation of exon 8 of *DPY19L2* gene in total globozoospermic patients referred to Royan Institute

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Introduction: Lack of acrosome decreases the capacity of the sperm for penetrating in the oocyte which consequently results in infertility. Globozoospermia is a rare and severe teratozoospermia characterized by round-headed spermatozoa lacking acrosomes. *DPY19L2* is one of the genes which are dominantly expressed in the testis and it has been shown that is involved in the cause of this phenotype. Recent studies have shown that in large majority of globozoospermia patients a 200 kb deletion including *DPY19L2* gene occurs. Different mutations in exon 8 of this gene have been also observed.

Materials and Methods: In total 24 men with total globozospermia and 24 men with normal spermogram referring to Royan Institute were selected. Then we sequenced exon 8 and intron boundaries in the non-deleted patients using specific primers and PCR technique.

Results: In our results no mutations were detected in exon 8 of the patients without the gene deletion, which were 29.17% of the total globozospermia patients.

Conclusion: According to our data, exon 8 mutations or polymorphisms has no effect on globozospermia but whereas 70.83% of globozospermia patients had whole

DPY19L2 deletion and on the critical role of DPY19L2 protein in acroplaxome attachment to the nucleus, it can be concluded that the absence of this protein is one of the major causes of globozoospermia in Iranian infertile men. In addition, exploring other coding exons of this gene is our next aim.

Key words: *DPY19L2 gene, Globozoospermia, Male infertility.*

P-68
Anti-oxidant effects of herbal supplements on seminal parametmeters

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Introduction: Varicocele is one of the leading causes of male infertility, causing a decrease in sperm parameters such as motility, count, concentration, and abnormal sperms such as azoospermia and asthenospermia.

Materials and Methods: A herbal compound capsule containing 4 herbs was prescribed 5 times perday (3 times during morning and evening and 2 times at night), and because of the warm nature of these herbs, 3 herbal extracts including Cichorium intybus L, Salix Aegyptiaca and Fumaria Parviflora 3 cups/day were added to this medication.

Results: After 4 weeks of consuming these medications, the body warmness decreased , ejaculated sperm volume and other sperm parameters increased including motility, concentration and morphology of sperm changed form astenia and oligospermia to normal morphology.

Conclusion: The herbal compound containing antioxidant supplements and enriched in minerals and fat soluble vitamins showed promising effects on improving sperm parameters and increase the chance of male fertility.

Key words: *Varicocele, Increased sperm motility, Increased sperm count, Sperm paramteters, Antioxidants.*

P-69
The effect of brown algae Sargassum extract on freezing of sperm

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Introduction: Oxidative stress process resulting from freezing and thawing of sperm, influences parameters of sperm and decreases its fertility. Antioxidants play a conservative role against oxidative damages during freezing of sperm. Using an appropriate freezing bank effects on fertility and motility of sperm, so we decided to evaluate effect of antioxidant extract of brown algae Sargassum on reactive oxygen species (ROS) and parameters of frozen sperm.

Materials and Methods: 11 normal semen samples were divided in to 3 groups, including A treated with 250 µg/ml and B with 500 µg/ml of extract of algae Sargassum and C group without any treatment (as a control group) then froze throughout rapid freezing method. Using CASA software and oxisperm kit, the motility/morphology and ROS levels of sperm were measured, respectively. Finally data were analyzed with SPSS software assumed significant level of $p<0.05$.

Results: The analysis of sperm parameters demonstrated that general motility ($p=0.006$) and advanced motility ($p=0.007$) significantly increased in both A and B groups, after treatment compared with C group. Moreover, the level of ROS notably declined in both treated group. However this treatment revealed any changes in sperm morphology.

Conclusion: The brown algae Sargassum extract is rich in antioxidant component that leads to decrease oxidative damage via neutralizing of ROS and improve sperm motility. Our results suggested that the extract can be used as a potential antioxidant factor in sperm freezing bank.

Key words: *Sperm motility, Antioxidants, Reactive oxygen Species, Brown algae sargassum.*

P-70
Immunohistochemical evaluation of a testis-specific histone demethylase, JMJD1A, in tissue samples of infertile men referred to Royan Institute

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Introduction: Post-meiotic stages of mammalian spermatogenesis require unique and dynamic epigenetic events leading to histone removal followed by chromatin condensation. Through these events, histone demethylases such as JMJD1A play an important role in compaction of sperm chromatin due to regulation of histone methylation dynamics and alteration of chromatin structure. As "histone methylation" is one of the best-characterized modifications in the study of germ cell development, evaluation of presence/ absence

of JMJD1A protein as in impaired spermiogenesis were aimed in this study.

Materials and Methods: For this respect, consent was obtained from azoospermic infertile men referred to Royan Institute according to local ethical approval, and then testis tissue samples were collected from three groups including complete maturation arrest, Sertoli cell only syndrome, and hypo spermatogenesis as positive control. Immunohistochemical analysis of paraffin embedded tissue samples was performed qualitatively using anti-JMJD1A antibody to elucidate presence/ absence of this protein in nucleus of germ cells.

Results: Immunohistochemical analysis data showed absence of JMJD1A protein in nucleus of germ cells in groups with spermatogenesis impairment (complete maturation arrest and Sertoli cell only syndrome groups) compared to control group.

Conclusion: It can be concluded that there is an obvious association between absence of histone demethylation as a chromatin condensing state with impairment of spermatogenesis and male infertility.

Key words: *Spermatogenesis, Male infertility, Epigenetic, JMJD1A, Demethylation.*

P-71

Pregnancy outcome of intracytoplasmic injection with epididymal and testicular sperm retrieval in azoospermic patients

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Introduction: Intra cytoplasmic sperm injection (ICSI) is an assisted reproductive technique for treatment of infertility in azoospermic men. It seems that pregnancy outcome in ICSI following percutaneous epididymal sperm aspiration (PESA) is better than using testicular sperm retrieving from testis due to greater motility and better morphology of epididymal spermatozoa. To determine clinical pregnancy outcome following ICSI with epididymal sperm comparing to that of testicular sperm in men with azoospermia.

Materials and Methods: 60 men with azoospermia who were candidate for ICSI have been selected. Sperm retrieval was performed using PESA (n=30) or testicular sperm extraction (TESE) (n=30). The number of embryos and live births were analyzed and evaluated between two mentioned groups and also thawed sperms after freezing in TESE group (n=30).

Results: No difference was seen in age and duration of infertility between groups. The number of embryos were not different significantly between TESE and PESA groups ($p>0.05$), but the different was significant between PESA and TESE freeze groups (3.67 ± 2.89 vs. 2.10 ± 1.53 respectively; $p<0.05$). The live birth rate was higher in the PESA group compared with the TESE group $p<0.05$).

Conclusion: Intracytoplasmic sperm injection using sperm from epididymis is more effective than testicular sperm injection and can successfully be performed to treat men with azoospermia.

Key words: *ICSI, TESE, PESA, Freeze, Live birth rate.*

P-72

Prevalence and risk factor of congenital malformation in Kashan

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Introduction: Congenital malformation (CM) is a major childhood health difficulty. Treatment and rehabilitation of children with congenital malformations is costly and complete recovery is usually impossible. The prevention and treatment of congenital malformations are basic concerns for child health. The purpose of this study was to define rate of CM in Beheshti Hospital in Kashan, Iran to find out if there has been any variation in the rate and types of CM in this area.

Materials and Methods: This descriptive-observational study carried on 2700 births delivered from Beheshti Hospital in Kashan during 2012 and determines the prevalence of CM and type of it in this city.

Results: Prevalence of CM was 1.851% (2% in male and 1.61 % in female). Out of the 50 cases, 28 (56%) were males and 21 (42%) were females and 1 with uncertain genitalia. 13 members of the family were CM positive in musculoskeletal system (26%). Overall, anomalies of the cardiovascular system were second in frequency which involved 10 out of 50 patients (20%), also Down syndrome and skin malformation were the lowest rate anomaly in this study in Kashan (4% and 2% respectively). There wasn't statistical difference between prevalence of CM and neonatal gender and mother's age, father's age, mother's number of abortion, and mother's number of live children. But there was statistical difference between prevalence of CM with gestational age and Apgar number.

Conclusion: In this study the overall prevalence of congenital malformation among the newborn was less than those previous reported in Tehran and Yazd and higher than the rate of malformation in Gorgan and Arak. This difference determining the needs of more extensive studies.

Key words: *Congenital malformation, Newborn, Beheshti Hospital, Kashan.*

P-73

Relevance between testicular tissue vitrification and short term culture with degeneration and apoptosis genes expression

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Introduction: Cryopreservation of testicular tissue has been recommended as a promising technique for fertility preservation in pre-pubertal boys who scheduled to undergo gonadotoxic treatment. Our aim was finding the quota of apoptosis genes involved in extrinsic pathway in testicular cell death after vitrification and during short term culture.
Materials and Methods: Testes were obtained from 7 days old NMRI male mice, divided and randomly distributed into control and vitrification groups. Vitrification was performed in 3 step by increasing concentration of vitrification solution (DMSO, EG). Both fresh and vitrified-warmed testes was cultured in RPMI and 10% KOSR for 20 hr. Real-time PCR, flow cytometery and light microscopy were used respectively for evaluation of gene expression, cell death and tissue integrity at 0, 3 and 20 hr of culture.
Results: Decreasing of tissue integrity was obvious in vitrification group as compared to the control one at all times of culture. Mean percentage of cell death was significantly ($p<0.05$) higher in vitrification group in comparison with control during culture period. Although expression of Fas was significantly ($p<0.05$) higher in vitrification group at 0 and 3 hr of culture, it was significantly lower at 20 hr of culture as compared to the control group. Significant ($p<0.05$) increase of Fas ligand was found in vitrification group at 3 and 20 hr of culture. Mean percentage of Caspase 3 was significantly ($p<0.05$) higher in vitrification group than control group.
Conclusion: Concurrent increment of cell death and apoptosis genes expression in vitrification group during culture period, could be a reason for extrinsic pathway involvement in degeneration of testis tissue after vitrification and also during culture period.
Key words: *Extrinsic pathway of apoptosis, Tissue degeneration, Vitrification, Short term culture.*

P-74
Magnetic activated cell sorting and its application for selection of human non apoptotic spermatozoa in ART

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Introduction: Hydrogen peroxide has been implemented in literature to induce a significant increase in caspase. Activation of caspase 9 triggers a cascade of caspase activation, including caspase 3, which promotes cellular apoptosis. Magnetic cell sorting (MACS) using annexin V-conjugated microbeads eliminates apoptotic spermatozoa with annexin V-positive.
Materials and Methods: A total of 20 semen samples were obtained from male partners of couples for analysis. One aliquot (0.5ml) of the sperm suspension was subjected to MACS. Motility and concentration was checked, and a sample taken for tunnel before and after MACS. The remaining sperm suspension was divided into 6 tubes (2x control, 2x peroxide, 2x peroxide/ melatonin). DNA fragmentation was evaluated using the TUNEL assay (Roche, Indianapolis, IN, USA) (14), with some modifications. Data were analyzed using Graph Pad Instat Ver. 3.10.
Results: Results of the TUNEL assay in pretreatments of human spermatozoa with 100uM peroxidase for 24 hrs revealed that the percentage of sperm with fragmented DNA was significantly lower on the sored sperm after sorting MACS ($p<0.001$ vs. control). Following the pretreated human spermatozoa with peroxidase for 24 hr, percentage of sperm motility and progressive motility were significantly reduced ($p<0.001$ vs. control). Pretreatments of human spermatozoa for 24 hr revealed that the percentage of sperm motility and progressive motility were significantly reduced by 100 µM peroxidase and peroxidase with MACS, versus zero hour in control group ($p<0.001$).
Conclusion: The sperm was treated with 100 µM peroxidase sorting using MACS retain appropriate spermatozoa and select sperm good quality. The use of MACS will select only sperm with intact this result in high percentage motility and progressive motility.
Key words: *Apoptosis, Human spermatozoa, MACS.*

P-75
Bibliometric mapping and clustering analysis of Iranian-based research on reproductive medicine

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Introduction: Nowadays infertility is a major problem in the world. Increasing the rate of infertility in the world and Iran led our study to assess the trends in Iranian research output related to reproductive medicine through the year of 2010-2014.
Materials and Methods: We used bibliometric mapping and clustering analysis method to visualize representing bibliographic data of Iranian production. All publication data from Scopus was retrieved for 2010-2014.
Results: Analysis of data showed that a total number of 3035 papers had been indexed in Scopus through the period of study.

Conclusion: The study indicated that scientific production in the field of reproductive medicine had increased in the past 5 years.

Key words: *Reproductive medicine, Bibliometric analysis.*

P-76

Menstrual pattern following tubal ligation: A historical cohorts

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Introduction: Tubal ligation (TL) is recommendable for women completed their family. The existence of the menstrual disorders following this procedure has been the subject of debate for decades.

Materials and Methods: A historical cohort study was carried out on 140 women undergoing tubal ligation and on 140 women who used condom as the main contraceptive method. They aged between 20 and 40 years and were selected from a health care center, during 2013-2014. The two groups were comparable in demographic and personal characteristics. Data collection tool was a questionnaire including questions regarding demographic, menstrual and obstetrical characteristics. A validated Pictorial Blood Loss Assessment Chart (PBLAC) was also used to measure the menstrual blood loss. All statistical analyses were carried out using software package used for statistical analysis (SPSS) version 20 (SPCC Inc., Chicago, IL, USA). Student's t-test and chi-square test were carried out to reveal the statistical differences between the groups. Logistic regression was done to build a prediction model in menorrhagia.

Results: Women with TL had more menstrual irregularity than those without TL (24.3% vs. 10% respectively, p=0.002). Women with TL had more polymenorrhea (9.3% vs. 1.4%; p=0.006), hypermenorrhea (12.1% vs. 2.1%; p=0.002), menorrhagia (62.9% vs. 22.1%; p<0.0001) and menometrorrhagia (15.7% vs. 3.6%; p=0.001) than those without TL. There is a significant difference in the PBLAC score between women with and without TL (p<0.0001). According to logistic regression, age (OR=1.08, CI:1.07-1.17; p=0.03), TL (OR=5.95, CI:3.45-10.26; p<0.0001), and cesarean section (OR=2.72, CI:1.49-4.97; p=0.001) were significantly associated with menorrhagia.

Conclusion: We found significant differences in menstrual disorders between women with and without TL. Therefore, women should be informed by the health providers regarding the advantages and disadvantages of TL before the procedures.

Key words: *Historical cohort study, Tubal ligation, Menstrual disorders, PBLAC.*

P-77

Effect of nano silver on morphological and chromosomal abnormality of NMRI mouse fetus

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Introduction: Development of nanotechnology caused the use of the materials in nano sizes. Nowadays, special biological properties of nano silver are playing an important role in our life. Some studies suggest a potential for adverse effects of nano silver on fetal development of mammals, but additional research is needed. In this study we decide to evaluate the effects of nano silver on fetus chromosomal structure and its development.

Materials and Methods: 24 pregnant mice were divided into four groups. Nano silver (1 mg/kg, 70 nanometer) were gavaged to the first, second and third group, from the 1^{th}-7^{th}, 8^{th}-14^{th} and 1^{th}-14^{th} gestational days respectively. Nothing was gavaged to the control group. On 14^{th} day the pregnant mice were dislocated and the liver of fetus was used for karyotyping analysis. The fetuses were weighed and their crown-rump length and head circumference were measured by caliper.

Results: According to the effects of nano silver, the results indicated that body weight of embryos were significant decreased in third group in comparison with the others (p<0.001). Also there were significant reduction of crown-rump length of fetus in third and second group compared to other groups (p<0.05). There was lower fetus head circumference among nano silver treated and controls (p<0.05). Karyotyping analysis of fetal liver was normal in all groups.

Conclusion: It seems that abuse of nano silver during pregnancy can reduce weight, crown-rump length and head circumference in mice fetus, but its mechanism is not completely clear.

Key words: *Pregnant mice, Nano silver, Karyotype.*

P-78

Neuronal markers expression of induced human adipose-derived stem cells in alginate hydrogel

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Introduction: Hydrogels provide appropriate three-dimensional environment for cell cultures and cell encapsulation in hydrogels is a promise plan for tissue engineering applications. Alginate is a biocompatible hydrogel that provides a supportive system for the encapsulated cells. Moreover human adipose derived stem cells (hADSCs) might be a suitable source of cells for use in autologous cell therapy; also these cells can differentiate into neuron-like cells. Therefore, in this study we evaluated effect of alginate hydrogel on the neural potential of induced hADSCs.
Materials and Methods: Isolated hADSCs were induced in neural medium and encapsulated in alginate hydrogel. Using Immunocytochemical and real-time RT-PCR analysis, some neural markers were evaluated in differentiated hADSCs.
Results: The expression of Nestin, GFAP and MAP2 markers significantly increased in alginate cell cultures relative to monolayer induced cells ($p<0.001$).
Conclusion: These findings showed that alginate hydrogel can provide a suitable environment for neural differentiation of hADSCS.
Key words: *Human adipose-derived stem cells, Alginate hydrogel, Neural differentiation, Tissue engineering.*

P-79
Protective effect of N-acetyl-L-cysteine on testicular tissue in mice treated with Para-Nonylphenol

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Introduction: Para-Nonylphenol (p-NP) is an alkylphenol considered as an environmental pollutant with estrogenic and toxic effects. It can also cause morphological and functional alterations in the male reproductive system inducing infertility. The purpose of this study was to investigate the protective effects of N-acetyl-L-cysteine (NAC), as a powerful antioxidant, on p-NP-induced testicular toxicity in mice.
Materials and Methods: Adult male NMRI mice (32±4 gr) were divided randomly into 4 groups (n=6), control, NAC (150 mg/kg/day), p-NP (250 mg/kg/day) and p-NP+NAC, and they were treated orally for 35 days. Finally, mice were killed, their right testis were removed and fixed followed by sectioning, tissue processing and Heidenhain azan staining. Testicular tissue sections were then evaluated using stereological method. The level of Malondialdehyde (MDA), a lipid peroxidation index, was also measured in the testis of mice. Data were statistically analyzed using one way ANOVA and means were considered significantly different at $p<0.05$.
Results: A significant decrease in the mean total volume of testis, diameter, length and the volume of the seminiferous tubules, height of the germinal epithelium and the thickness of the basement membrane along with a significant increase in the MDA level was observed in the p-NP group compared to the control ($p<0.01$) while the above parameters were compensated to the control level in the p-NP+NAC group ($p<0.01$).
Conclusion: Our findings indicate that N-acetyl-L-cysteine can protect the testicular tissue against the tissue damage and oxidative stress induced by para-Nonylphenol in mice.
Key words: *Para-nonylphenol, N-acetyl-L-cysteine, Stereology, Testis.*

P-80
Effects of dietary canola oil on sperm quality parameters at Afshari Ram Breed

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Introduction: In mammalian and non-mammalian spermatozoa, there are natural fatty acids, cholesterol, phospholipids (mainly lecithin, cephalin and sphingomielin) and glycolipids. Phospholipids of mammalian sperm cell membranes characteristically contain very high proportions of long-chain polyunsaturated fatty acids, particularly n-3 series
Materials and Methods: 18 Kurdish rams were selected with weight average 54.47±2.58 kg and with the age of 3-4 years approximately. They were divided to two experimental groups randomly. Experimental groups were control and canola oil (2.5% of DMI). Before of study, 10 day was considered as adaptation period and then Sperm was collected by electro ejaculation at 6 week and 11 week after begging of adaptation period and sperm motility was analyzed by using CASA software at zero time and 24 hours post ejaculation. Also, percentage of live/dead sperm and morphology was evaluated by staining of Eosin-Nigrosin and Papanicolaou, respectively.
Results: The results showed that motility parameter wasn't significantly different between whole experimental groups at first time (week 6) but PM% and TM% was significantly different in canola oil group compare to control group at second time (week 11), separately. Percentage of live/dead sperm and morphology was higher significantly in canola oil group compare to control group.
Conclusion: It was concluded that canola oil can improve sperm motility, morphology and viability in Afshari ram.
Key words: *Canola oil, Motility, Afshari Ram, Sperm.*

P-81
Mutation analysis of bone morphogenetic protein-15 gene in Iranian patients with polycystic ovarian syndrome

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Introduction: With the prevalence on the order of 6-10%, polycystic ovarian syndrome (PCOS) is considered the most common endocrinologic disorder affecting women in their reproductive age. Major criteria that have been proposed for the diagnostic characteristics of PCOS are clinical and/ or biochemical hyperandrogenism, ovulatory dysfunction and polycystic ovary in ultrasound. It has been suggested that genetic factors participate in the development of PCOS. Follicular development has been considered as one of the impaired processes in PCOS. *BMP-15* gene is a candidate gene in follicular development and its variants may play role in pathogenesis of PCOS. Previous investigations have revealed controversial results on *BMP-15* mutations in PCOS women among various racial groups.

Materials and Methods: A cross-sectional study was carried out on 70 PCOS patients. Following taking the informed consent, 5ml venous blood was taken from each participant. Genomic DNA was extracted from the blood sample by salting out Method. Then a set of PCR reactions for *BMP15* gene was performed using specific primers followed by genotyping with direct sequencing.

Results: As for exon1 in *BMP-15*, 20 heterozygote (G/C) and 2 homozygote (G/G) cases were found in 70 PCOS patients. Also, one -9 C>G polymorphism in 5'UTR and 3 cases of A308G mutation (A/G) were discovered in our patients.

Conclusion: Result of this study indicates that variants of *BMP-15* gene could be related to susceptibility of development of PCOS and may be used as genetic markers for detecting PCOS in the future.

Key words: *Polycystic ovarian syndrome, Bone morphogenetic protein 15.*

P-82

Effect of adding human chorionic gonadotropin to frozen thawed embryo transfer cycles with history of thin endometrium

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Introduction: Embryo implantation process is a complex phenomenon and depends on fetal and maternal factors interaction. Endometrial thickness is needed for successful implantation. Increasing endometrial thickness, raise the chance of clinical pregnancy. The triple line pattern, with thickening more than 7mm, is indicating the greater chance of successful implantation. We designed this study to assess adding HCG to the conventional protocol in endometrial preparation in women with thin endometrium and history of IVF-ET failure.

Materials and Methods: This non-randomized clinical trial study (quasi experimental design) was performed in Yazd Research and Clinical Center for Infertility on 28 patients. Participants were women who candidate for frozen thawed embryo transfer and had 2 previous failed ET cycle because of thin endometrium. All patients received 8 mg estradiol valterate on second day of menstrual cycle and continued during the study. HCG was administrated (150 IU, IM) from 8th days of cycle. In 12th-13th day Trans-vaginal sonography was done, when endometrial thickness reached at least 7mm, HCG was discontinued and frozen thawed embryo transfer was done.

Results: Totally 28 patients were included. The mean age of participants was 30.39±4.7 years. The mean of endometrial thickness before and after HCG were 5.07±0.43 and 7.85±0.52 mm which were significantly different (p=0.00). After HCG administration 100% patient's endometrial thickness reached more than 7mm. The frequency of 20% improvement after HCG was 89.3% (25 patients). Also there were 5 (17.8%) clinical and chemical pregnant women after HCG.

Conclusion: Our study findings suggest that adding HCG to the conventional preparation method is an effective protocol and significantly improved endometrial thickness and pregnancy outcomes in women with previous embryo transfer failure because of thin endometrium.

Key words: *Human chorionic gonadotropin, Frozen thawed embryo transfer, Thin endometrium.*

P-83

Glycyrrhiza glabra and vitamin C can reduce toxic effects of acrylamide on sperm parameters in rat

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Introduction: Acrylamide (AA) is a chemically reactive substance used in various industries. Recently, acrylamide was discovered in a variety of human foods including heat-processed starchy foods such as potato chips and bread. AA is able to induce sperm damage in male mice.

Materials and Methods: In this experimental study, 30 male Wistar rats of 28 days of age were divided in to five groups: Acrylamide, Acrylamide+ Vitamin C, Acrylamide+ Glycyrrhiza glabra, Acrylamide+ Vitamin C+ Glycyrrhiza glabra and Control. All treatments were administered (oral Acrylamide 10 mg/kg, Vitamin C 200 mg/kg and Glycyrrhiza glabra 150 mg/kg) daily for two months. Thereafter, the cauda epididymis of each rat was dissected and placed in 1 mL of pre-warm Ham's F10 culture medium for 30 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability.

Results: The results showed that almost all of the sperm parameters except non progressive motility were significantly different between groups (p=0.001). Also, the mean of sperm parameters in Acrylamide+ Vitamin C+ Glycyrrhiza glabra group was higher than other groups.
Conclusion: This study showed that the co-administration of vitamin C and Glycyrrhiza glabra as antioxidant can reduce the detrimental effects of acrylamide on sperm parameters in rats.
Key words: *Glycyrrhiza glabra, Vitamin C, Acrylamide, Sperm, Rat.*

P-84
Infertility in male by neurological disorders

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Introduction: Normal sexual and reproductive functions depend largely on neurological mechanisms. Neurological defects in men can cause infertility through erectile dysfunction, ejaculatory dysfunction and semen abnormalities.
Materials and Methods: Science Direct, PubMed, Cochrane, CINAHL, Embase, ProQuest Dissertations, Scopus, (2000-2015) were searched for English-language studies using a list of keywords. The books about physical therapy and medical and neurological were studied too.
Results: Among the major conditions contributing to these symptoms are pelvic and retroperitoneal surgery, diabetes, congenital spinal abnormalities, multiple sclerosis and spinal cord injury. Erectile dysfunction can be managed by an increasingly invasive range of treatments including medications, injection therapy and the surgical insertion of a penile implant. Retrograde ejaculation is managed by medications to reverse the condition in mild cases and in bladder harvest of semen after ejaculation in more severe cases.
Conclusion: An ejaculation might also be managed by medication in mild cases while assisted ejaculatory techniques including penile vibratory stimulation and electro ejaculation are used in more severe cases. If these measures fail, surgical sperm retrieval can be attempted. Ejaculation with penile vibratory stimulation can be done by some spinal cord injured men and their partners at home, followed by in-home insemination if circumstances and sperm quality are adequate. The other options always require assisted reproductive techniques including intrauterine insemination or in vitro fertilization with or without intracytoplasmic sperm injection. The method of choice depends largely on the number of motile sperm in the ejaculate.
Key words: *Ejaculation, Electro ejaculation, Infertility, Nervous system diseases.*

P-85
Adenosine deaminase G22A polymorphism and recurrent spontaneous abortions

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Introduction: Adenosine deaminase (ADA) is an enzyme of purine salvage pathway and has two important isoenzymes ADA1 and ADA2. The adenosine deaminase G22A polymorphism (ADA*2) increases the level of adenosine. Adenosine may play a protective role against recurrent spontaneous abortions (RSA), since it regulates blood flow into the uterus and placenta.
Materials and Methods: A total of 100 women were classified in two groups: First group, with a history of recurrent spontaneous abortions (n=50), and second one, without a history of abortions (n=50). Genomic DNA was extracted from peripheral blood with a commercial kit and PCR-RFLP analysis was used to identify the G22A genetic polymorphism.
Results: The frequency of homozygotes (AA) was 2% in control group, whereas no homozygote (AA) was found in the case group ($p>0.05$). The frequency of heterozygotes (AG) was 20% in the control group and 8% in the case group ($p<0.05$). The frequency of homozygotes (GG) was 78% in the control group and 92% in the case group ($p<0.05$). A significant increase in the frequency of AG genotype in controls ($p<0.05$, OR=0.348) relative to women with the history of RSA demonstrates the protective effect of AG genotype in controls.
Conclusion: The data suggest that women carrying the ADA*2 allele which is associated with the lower enzymatic activity are better protected against recurrent spontaneous abortions. Because decreased enzymatic activity increases adenosine levels. High adenosine levels may play a protective role against recurrent spontaneous abortions, since it regulates blood flow into the uterus and placenta.
Key words: *PCR-RFLP, Recurrent spontaneous abortion, Adenosine deaminase.*

P-86
Seminal bacterial contaminations: Probable factor in unexplained recurrent pregnancy loss

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Introduction: It is estimated that about 50% of causes of recurrent pregnancy loss (RPL) cases remain unknown. Sperm factors are suggested to have probable

role in cases with RPL. The goal was to determine the possible relationship between semen bacterial contaminations with unexplained RPL. Also, the correlation between number of bacterial colony and sperm chromatin condensation was examined.

Materials and Methods: This study consisted of 30 fertile men (group A) and 30 infertile men (group B) with unknown RPL in their wives. Semen collection and analysis were done according to WHO manuals. Sperm count and motility were evaluated by Makler chamber. Eosin-Nigrosin and Papanicolaou staining methods were applied for viability and morphology assessment, respectively. The semen samples from both groups were cultured for aerobic bacteria. Aniline blue (AB) and toluidine blue (TB) staining methods were applied for evaluating sperm chromatin condensation.

Results: The numbers of colonies were significantly higher in group B when compared to group A. Also, S. aureus and E. coli contaminations showed significant differences between two groups. Both AB+ and TB+ sperm cells showed significant increase in group B compared to group A. There was a significant negative correlation between colony number and progressive motility (p=0.01), and sperm viability (p=0.007). In addition, positive correlations were found between colony number and AB+ (p=0.001) and TB+ (p=0.004) as well.

Conclusion: Bacterial contaminations in semen of men from RPL couples had significantly higher levels when compared to fertile controls. Presence of microorganisms in semen may be correlated with irregular sperm parameters and quality.

Key words: *Recurrent pregnancy loss, Bacteria, Semen.*

P-87
Precedent infertility and attitudes towards preferred Cesarean

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Introduction: Prevalence of natural delivery and caesarean section in pregnant women of a country is one of the indicators of health system performance. Currently, we are exposure to increasing elective Cesarean with different reasons and attitudes.

Materials and Methods: In this descriptive-analytical study, 271 pregnant women completed a researcher-made questionnaire including, demographic characteristics (age, precedent of infertility) and 10 attitude questions about the influencing factors on choosing Cesarean, based on five Likert scale which was a score of 10-30 negative attitude (opposite preference for Cesarean) and 31-50 positive attitude (positive preference for Cesarean). Therefore, to analyze the obtained data, Chi-square test was used through applying SPSS19 software.

Results: Overall 74 subjects (28%) had negative attitudes, while 192 subjects (72%) had positive attitudes. In total 7 subject of pregnant women group (2.6%) had a precedent of infertility and 259 pregnant women had not (95.6%). During the study, 5 subjects did not answer questions. In addition, 5 subjects with precedent infertility (73.4%) vs. 69 subjects (26.6%) with no precedent, showed negative attitudes. 2 subjects (26.6%) vs. 190 pregnant women (73.4%) had positive attitude toward preference of Caesarean section. There was a significant relationship between precedent infertility and attitude.

Conclusion: According to the results, women with precedent infertility who are using assisted reproductive techniques, are more aware of advantages and disadvantages of different methods of termination of pregnancy and most of them opposed natural delivery. Therefor giving them information through related groups of women's health, can change their attitudes.

Key words: *Precedent infertility, Attitude, Preference cesarean.*

P-88
Protective effects of BDNF against oxidative damage and apoptosis in human spermatozoa

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Introduction: Oxidative Stress (OS) is a condition when there is an improper balance between the amount of pro-oxidant substances and the amount of seminal plasma antioxidant factors that can be induced by repeated cycles of centrifugation and seminal plasma antioxidants, removal during the sperm preparation in Assisted Reproductive Technology (ART) Reactive Oxygen Species (ROS) can cause decrease of motility, increase of apoptosis and impaired sperm function, and ultimately, influence on fertilization and pregnancy rate. Therefore, currently there is a great interest to the use of antioxidants to prevent ROS generation and ROS-induced apoptosis during the sperm preparation processes. Brain-derived neurotrophic factor (BDNF) is member of neurotrophin family that has anti- oxidant and anti-apoptotic effects on nervous system. Recent researches show that it also plays key role in male and female reproductive system such as spermatogenesis, nuclear and cytoplasmic maturation and embryo development.

Materials and Methods: Liquefied semen samples obtained from normozoospermic men (n=25) after preparation by double wash (400× gr, 5 min) swim-up technique were divided in to two groups of control and treated group (with BDNF). Then motility was

evaluated by means of CASA. Reactive oxygen species (ROS) and apoptosis were assessed by flowcytometry.
Results: Addition of BDNF to the sperm media significantly increased mean total motility and progressive motility (75.54±2.63% vs. 66.81±2.48%, p<0.001, and 46.18±2.2 vs. 39.631±1.97%, p<0.002, respectively). Also BDNF treatment caused decrease of H_2O_2 (50.01±6.08 vs. 38.31±5.250, p<0.02), necrosis (23.26±2.71 vs. 27.52±2.27, p<0.001) and apoptosis (3.26±0.4 vs. 3.76±0.55, p<0.49).
Conclusion: Considering this result, BDNF treatment can be a potential tool against oxidative damage and apoptosis in human spermatozoa.
Key words: *Brain-derived neurotrophic factor (BDNF), ROS, Apoptosis, Flowcytometry.*

P-89
Iranian couple's sexual compatibility during the time: A qualitative study

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Introduction: Since sexual compatibility is facilitators for marital compatibility and the root of many divorces is the sexual incompatibility, and due to the limited data available in this topic, this qualitative study was designed to exploring the changes of Iranian couple's sexual compatibility during marriage years.
Materials and Methods: In-depth interview and written narrative methods were used. Purposive sampling with maximum variation (age, education, years of marriage, number of children, etc.) was conducted. Ale Yasin Clinic in Tehran and Fatemieh Clinic in Varamin were the setting of research. Times of interviews were almost 40 min. Data reached to saturation after 14 semi-structured depth interviews and 54 written narratives with the 68 married men and women. Conventional content analysis was done in MAXQDA10 software. Lincoln and Guba criteria were used to validate qualitative data.
Results: Emerged categories included: "duration to reach sexual compatibility" and "mainly increasing sexual compatibility during the time" and "rarely decreasing sexual compatibility during the time". Couple's sexual compatibility increases over time due to "Getting to know each other more", "increasing sexual awareness and experience", "talking about sex with spouse", "skill acquisition for mutual pleasure and satisfaction". Couple's sexual compatibility may decrease over the time because "children growing and conflict parental role with spousal role "and "financial difficulties".
Conclusion: With knowing the impact of passing the time on couple's sexual compatibility many divorces can be prevented by suitable planning and counseling.
Key words: *Sexual compatibility, Iranian couples, Qualitative study.*

P-90
Effect of non-polar extract of Phaleria macrocarpa (Mahkota Dewa) on spermatogenesis in rats

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Introduction: Fertility is the natural capability of giving live. In male, fertility requires the production of large numbers of normal and mature spermatozoa by testes through a complex process call spermatogenesis. Evidence shows increase male age is associated with the decline of mature sperm in seminiferous tubule. Aqueous extract of Phaleria macrocarpa (PM) has been shown to improve fertility in rats by increasing the spermatogenesis.
Materials and Methods: Hexane extract of Phaleria Macrocarpa was prepared by extracting grinded dried slices of fruit followed with drying of the extract using rotary evaporator. A total of 30 male rats were divided into 5 different groups and subjected to daily treatments with PM extract at one of the following concentration: 0, 6, 12, 60 mg/kg of hexane extract, or commercial testosterone hormone for seven consecutive weeks. They will be fed with pellet once a day in the morning and drinking water will be given ad libitum. Body weight was measured once a week. On the last day of supplementation period, the rats were sacrificed and the testis was isolated for histological evaluation.
Results: The result showed that no significant effect of non-polar extract of PM on testes size and volume, thickness of seminiferous tubules and spermatogonia cell number. The mean thickness of seminiferous tubules were 62, 68, 62, 60, 60 µm for 60, 12, 6, 0 mg/kg of PM extract and commercial testosterone respectively. The mean for spermatogonia cell count in rats treated with 60, 12, 6 and 0 mg/kg and commercial testosterone were 52.8, 43.8, 47.0, 52.6, 52.0 cells respectively.
Conclusion: The study concludes that the non-polar extract of PM has no effect on the fertility of male.
Key words: *Phaleria macrocarpa (PM), Spermatogenesis, Rat.*

P-91

The functional SNP analysis of *CYP2D6* gene in patients with Polycystic ovary syndrome (PCOS)

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Introduction: PCOS is a common ovulatory disturbance, which influents about 5-10% women of reproductive age. Clomiphene citrate is used for ovulatory induction in PCOS, that is metabolized by Cytochrome P450 2D6 (CYP2D6). More than 120 variable alleles have been reported for CYP2D6 that some of them are poor metabolizer and cause resistance to drugs that are substrates of CYP2D6.
Materials and Methods: Whole blood was collected from drug control, PCOS and fertile women (60 in each group). RFLP method was used to determine *CYD2D6*34* (2850C>T). Patients should have the Rotterdam criteria. Inclusion criteria's were age under 35 years old, candidates for IUI, not using other drugs and not have ovary cautery.
Results: Genotype distribution of CC, CT and TT in PCOS group was 47.4%, 40.4% and 12.3% respectively (p=0.479), and frequency of these genotypes in drug control group was 36.7%, 50% and 13.3% respectively (p=0.479), which was very close to results in fertile group: 33.3%, 56.7% and 10% respectively. The number of antral follicles (follicle diameter≥15mm) was measured as drug response which was 1.21 (p=0.118) in clomiphene citrate administered PCOS patients (64%) while this was 1.64 (p=0.09) in 36% of patients treated with letrozole (follicle diameter≥15mm). Same results were observed in drug control group: antral follicles were 1.66 in 80% of clomiphene citrate treated whereas it was 1.77 when they received letrozole (20% of patients).
Conclusion: Although genotype frequencies of 2850C>T polymorphisms were not significant between three groups, we observed cases bearing this polymorphism, showed resistance to clomiphene citrate, in both groups, which is the first time to report.
Key words: *CYP2D6, Polycystic ovary syndrome, Clomiphene citrate.*

P-92
Does L-carnitine therapy add any extra benefit to standard inguinal varicocelectomy in terms of deoxyribonucleic acid damage or sperm quality factor indices: A randomized study

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Introduction: Varicocelectomy and anti-oxidant therapy are both documented to have positive effect on spermatogenesis and improving semen quality.
Materials and Methods: 100 patients enrolled in this study and were randomly divided into 2 groups (50 patients in each group). In group 1, standard inguinal varicocelectomy and, in group 2, standard inguinal varicocelectomy plus oral antioxidant therapy (oral L-carnitine, 250 mg 3 times a day) were performed for 6 months. For all patients, routine semen analysis and DNA damage test of spermatozoa (by 2 methods of terminal deoxynucleotidyl transferase dUTP nick end labeling and protamine damage assay) were performed at baseline and at 3 and 6 months postoperatively.
Results: In both groups, the improvement in semen analysis parameters and DNA damage was observed, but there was not any statistically significant difference between the 2 groups in these parameters, although the slope of improvement in DNA damage was slightly better in group 2 (that was not statistically significant).
Conclusion: We observed that addition of 750 mg of L-carnitine orally daily to standard inguinal varicocelectomy does not add any extra benefit in terms of improvement in semen analysis parameters or DNA damage.
Key words: *DNA damage, Varicocelectomy, L-carnitine therapy.*

P-93
Different haplotype frequency in *AKAP3* gene in Iranian patients with short tail sperm

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Introduction: One kind of sperm abnormalities that leads to men infertility is short flagella of sperms. In this defect, fibrous sheath and axoneme are disorganized, the sperms tail is short, the numbers of sperms in the semen fluid reduce and the sperms are immotile. A Kinas anchoring protein 3 (*AKAP3*) gene encodes a protein that is involved in the fibrous sheath structure, regulation of sperm motility and head-associated functions such as capacitation and the acrosome reaction. AKAP3 interacts with the regulatory subunit of Protein Kinas A via its dimerization/ docking domain. In the present study, 30 patients with short tail sperm defect and 40 males with normal spermogram referred to Royan Institute were enrolled as case and control groups respectively. The genetic variation in

exon 5 of *AKAP3* gene which encodes the functional domain of this protein was studied.

Materials and Methods: PCR- sequencing was done on extracted DNA from blood samples of control and patient groups.

Results: According to the results, four haplotype polymorphisms 1378 T>C, 1391 C>G, 1437 T>C and 1573 G>A were observed in all samples studied. These polymorphisms were all observed as the mutant alleles. 92% C allele and 8% G allele of 1391 C>G polymorphism has been reported in East Asia. In the present study, 100% G allele was observed in this polymorphism.

Conclusion: This difference in frequency of mutant allele can be due to different ethnic of Asian population and ours. 1391 C>G alternation is located on the outside of the AKAP3 binding domain to AKAP4 and seems that it cannot have a role in the interaction between these two proteins.

Key words: *Short tail sperm defect, Fibrous sheath, AKAP3 gene, PKA, Polymorphism.*

P-94

Study of genetic alterations of *STK11* in patients with polycystic ovary syndrome (PCOS) and their response to ovarian stimulation

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Introduction: PCOS is the main reason of anovulatory caused infertility. Metformin use for PCOS patients to reduces hyperinsulinemia and androgens production through activation of STK11. We aimed to study the associations between c.842C>T and c.996G>A polymorphisms and risk of PCOS susceptibility in women plus the relations of two polymorphisms and response to ovarian stimulation in PCOS patients.

Materials and Methods: Blood samples were collected from patients and drug control group (male infertility) and fertile group (60 in each group). For genotype analysis, we used RFLP method. We used Rotterdam criteria for patient selection. Inclusion criteria's were age below 35 years old, candidates for IUI, not using other drugs and not have ovary cautery. Our data shown that frequency distribution of CT, TT,CC genotypes in exon6, in PCO Patients was 1.7%, 0%, 98.3% respectively (p=0.236), these frequencies in fertile group was 5%, 0%, 95% respectively and in drug control group was 0%, 1.7%, 98.3% respectively (p=0.236). We have not detected c.996 G>A polymorphism in any groups.

Results: The mean of age and BMI were 26.31±3.68 years and 27 kg/m^2 in PCOS and control group. Most of PCOS patients (64%) were received clomiphene citrate and 36% used letrozole whereas 80% of drug control was treated by clomiphene citrate and 20% used letrozole. In both groups their responses to drugs was measured by the number of antral follicles (follicle diameter ≥15mm) by ultrasonography that was 1.64 in PCOS, p=0.09 and 1.77 in drug control treated with letrozole that were better than clomiphene citrate (1.21 in PCOS, p=0.118 and1.66 in control).

Conclusion: Our data did not find any significant differences between these polymorphisms and response to ART medicines in PCOS women. According to our study, PCOS patients had higher response to letrozole rather than clomiphene citrate.

Key words: *STK11, Polycystic ovary syndrome, Polymorphism.*

P-95

Effects of electromagnetic fields of magnetic resonance imaging on male mice fertilization

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Introduction: Currently, the use of electromagnetic waves in medicine, especially in diagnostic devices such as magnetic resonance imaging (MRI) has increased and many of its biological effects have been reported. The aim of this study was to investigate the adverse effects of 1.5T MRI on fertility and reproductive parameters of male mice.

Materials and Methods: 40 NMRI adult male mice were randomly divided into two groups of control and experimental. The mice in the experimental group were exposed to MRI at 1.5T for 36 min once a week for a period of 3 weeks. Then, in the 1st day and 35th day after the final exposure, 10 mice were used for IVF and 10 mice for In vivo studies. MRI effects on testis weight, the duration of pregnancy, the number of newborns, sperm count, and fertility were evaluated. The obtained data were analyzed using ANOVA and Tukey's tests.

Results: According to the present study, one day after MRI exposure, testis weight, sperm count, and the number of born children were significantly decreased in the experimental group ($p<0.05$). Moreover, a significant number of the embryos failed to develop to the blastocyst stage. In contrast, 35 days after exposure, no statistically significant difference was found ($p>0.05$).

Conclusion: Based on the results of the present study, it seems that although the MRI at 1.5T has adverse effects

on fertility and reproductive parameters of the adult male mice, these side-effects are reversible.
***Key words*:** *Electromagnetic fields, MRI, Sperm, Fertility.*

P-96
Interference of histone modification with aberrant expression of *HOXA10* gene in eutopic and ectopic endometrial tissues of women with endometriosis during menstrual cycle

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Introduction: Disruption of the balance of epigenetic networks, which involves DNA and histone modifications, can cause several pathologies, including reproductive disorders such as endometriosis. *HOXA10* gene expressed in endometrium plays an important role in uterine receptivity at the time of implantation, uterine organogenesis and functional endometrial differentiation.
Materials and Methods: Epigenetic analysis were assayed by Chromatin Immunoprecipitation (ChIP), using anti- H3K9ac, H3K9Me2, H3K27Me3, H3K4Me3 antibodies and quantitative expression analysis was performed by real-time PCR technique. For this respect, eutopic and ectopic endometrial samples were collected using laparoscopy from 36 women with documented endometriosis, and also endometrial biopsies were obtained from 22 healthy women with male factor problem as a control group. Ethical approval and informed patient consent was gained for the use of tissue samples.
Results: Data showed a harmonious pattern between mRNA expression of HOXA10 and epigenetic state of its promoter region, in the way that, in secretory phase the activating epigenetic marks, H3k9ac and H3k4me3 were higher in ectopic endometrial tissues and H3K9ac itself were lower in eutopic endometrium. In contrast, H3K9me2 and H3k27me3, the epigenetic marks, known to be associated with gene repression, showed a different pattern in which that, H3K9me2 were higher in eutopic endometrium and H3K27me3 were lower in ectopic endometrial tissues.
Conclusion: Our findings suggest epigenetic might be greatly responsible for aberrant expression of *HOXA10* gene in eutopic and ectopic endometrial lesions of patients with endometriosis.
Key words: *HOXA10 gene, Endometriosis, Menstrual cycle, Histone modifications, Epigenetics.*

P-97
Evaluation of action and mechanism of L-Carnitine in improved sperm quality in male infertility

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Introduction: About 15% of married couples are infertile and that approximately 50% of this is due to male factor infertility. A number of drugs have been proposed as being possible causes of male factor infertility. Estudies showed that a diet supplemented with L-carnitine can improve sperm quality in some mammalian species. In humans, 75% of carnitine derives from diet, while 25% is synthesized from lysine and methionine, although the enzyme that catalyses the hydroxylation of the 4-butirrobetain in L-carnitine is present in few tissues.
Materials and Methods: This review was conducted using the following databases: Medline, Pubmed, Scopus and Science direct from 1990-2014 for better understanding of the mechanism and action of L-Carnitine in male infertility.
Results: L-Carnitine can increase the concentration of this component in the epididymal tubules and spermatozoa. This molecule increases their motility and fertilizing abilities. Both free L-carnitine and acetylated L-carnitine can be accumulated in spermatozoa. In sperm cells, L-carnitine transports fatty acid to mitochondria for oxidation and production of energy for epididymal spermatozoa. Also L-Carnitine has antioxidant property; it via deleting excess acyl-CoA (formyl-CoA), due to toxic effect have important role in cellular detoxification. L-carnitine as antiaging protects cellular membranes against oxidative damage. It prevents protein oxidation, pyruvate and lactate oxidative damage. During sperm maturation, a reduction in total lipid content has been seen due to the changing composition of fatty acids in sperm cells. This leads to an increase in the fluidity of spermatozoa's plasmalemma. Moreover, L-carnitine reduces lipid availability for peroxidation which guards against potential peroxidative damage.
Conclusion: The result of studies showed that addition of L-carnitine to the diet of male infertility is effective in increasing semen quality, but these results need to be confirmed by more clinical trials.
Key words: *L-Carnitine, Sperm quality, Male infertility.*

P-98
Effects of cell phone radiofrequency electromagnetic waves (RF-EMW) on human spermatozoa

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Introduction: Cellular phones emit radiofrequency electromagnetic waves (RF-EMW) in the low frequency microwave. The detrimental effects of RF-EMW on the reproductive system and human fertility have been debated in recent years. The goal was to assess the influence of cell phones RF-EMW on different parameters and DNA integrity of human spermatozoa.
Materials and Methods: 50 semen samples were categorized in two groups: a) normospermia (n=24); and b) asthenozoospermia (n=24). After liquefaction, each sample was divided into two aliquots. One aliquot (experimental) was exposed to mobile radiation for 1 hr, and the second aliquot (unexposed) served as control. The sperm parameters and chromatin/ DNA integrity were examined and compared between groups.
Results: Normal samples exposed to RF-EMW showed insignificant differences in sperm motility, viability, and chromatin/DNA integrity. Regarding sperm morphology, normal morphology was significantly decreased in experimental group (p=0.04). Moreover, sperm parameters as well as DNA structure showed no significant differences in astenozoospermic samples.
Conclusion: Data did not show any noticeable impact of RF-EMW on human sperm quality. Further studies should be considered for confirmation of the results.
Key words: *Cell phone, Electromagnetic radiation, Sperm parameters, DNA integrity.*

P-99
Assessment of genetic variations in intron4 and exon5 of *RABL2B* gene in Iranian infertile men with immotile short tail sperm defect

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Introduction: The immotile short tail sperm (ISTS) defect, is a syndrome which causes male infertility. Patients with ISTS disorder have immotile short-tailed sperm with disorganized axonem, and a significant decrease in sperm counts too. Numerous proteins are involved in sperm tail formation. One of these proteins is RAB Like 2B (RABL2B), which recently its essential role in sperm intra-flagellar transport and fertility in male mouse has been demonstrated. So its gene, which called RAB Like 2B (*RABL2B*), is an appropriate candidate gene in human studies. RABL2B protein has 4 GTP binding domains which have important roles in protein function. Exon 5 of *RABL2B* gene, codes one of these main domains and intron 4 is the location for binding to some important transcription factors. The purpose of this study was to evaluate the genetic variations of exon 5 and intron 4 of *RABL2B* gene in infertile men with ISTS defect and controls
Materials and Methods: In this study, 30 infertile men with ISTS defect and 30 normozospermic men as controls were recruited. Remarkably it took 2 years to collect patients samples. To study the genetic variations, DNA was extracted from peripheral blood, then PCR sequencing was done.
Results: Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in exon 5, but an intronic variant (rs:144944885), was found in heterozygote form in one patient. No mutations or SNPs was identified in controls.
Conclusion: Although our data just revealed an intronic variations and no mutations or SNPs was detected in exon 5, due to the high expression of *RABL2B* gene in testis, and considering the fact that RABL2B is evolutionarily conserved and not many studies have been conducted about the exact role of this gene in human male fertility, evaluation of other exons and regulatory areas of this gene is strongly recommended.
Key words: *RABL2B gene, ISTS, Male infertility.*

P-100
Localization of septin 14 protein in sperm

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Introduction: In mammals, 14 septin genes have been identified so far. Disruption of septin functions has been implicated in the pathology of many diseases, including male infertility. Here, we study about one of the new members of the septin family called septin14 that is specially expressed in testis. This gene has two transcripts but only one of them transcript into proteins.
Materials and Methods: In this study, semen obtained from subjects attending Royan Institute that had normal spermogram. After that, immunocytochemistry, which is a common laboratory technique, was used to anatomically localize presence of a specific protein in cells by a specific primary antibody. The primary antibody allowed visualization of the protein under a fluorescence microscope when it was bound by a secondary antibody with a conjugated fluorescence.
Results: The protein expression was detected in the sperm from head to tail and highly localized in the front of the acrosome and the neck.
Conclusion: This is the first report on the localization of septin 14 in sperm. Septin 14 plays an important role in sperm morphology. Regarding the presence of this protein in sperm acrosome and neck, it can be concluded that the probable decrease of septin 14

protein expression in sperm may be associated with the pathogenesis of male infertility.

Key words: *Septin 14, Immunocytochemistry, Sperm, Male infertility.*

P-101
Molecular studying of sperm surface proteins and their cellular functions

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Introduction: Fertilization in mammalian is a complicated process with some connections among wide range of glycolipid, glycoprotein and antigenic indexes on sperm and egg surface of every species. We can improve our understanding in fertilization mechanisms and also get some information on molecular or immunological defects responsible for infertility by recognizing the antigens on sperm surface and investigating their role on cell physiological performance. The key challenge is to move from lists of identified proteins to informed understanding of biological function. Studying the sperm surface molecules and investigating their biochemical, biophysical and physiological properties to understand fertilization process.

Materials and Methods: A review of recent bibliography collected from internet database as PubMed, Google scholar, SID by the use of relevant keywords on different markers of cell surface and Sperm cell proteomic published in 2000-2014.

Results: Sperm membrane proteins contain an important part of membrane and are considered as special antigenic index to each species. Every change, increase or decrease in these proteins alters sperm performances and abilities. The important events which occurs during the interaction of sperm and egg and the reactions between them for fertilization relates to identification of these two cells by connecting receptors to ligands which exist on their cell membrane. Discovering the proteins on sperm surface which act as fertility biomarkers and participate in fertilization leads to more apprehension of this cell physiology and performance, so understanding molecular events like sperm acrosome reaction, capacitation and motility.

Conclusion: Sperm surface proteins can use as fertility biomarkers, therefore identification and supplementation these proteins to cryopreservation medium can improve post-thaw parameters of sperm and prevent sperm quality during cryopreservation.

Key words: *Proteomics, Sperm, Marker, Fertilization.*

P-102
Frequency of chromosome inversion (pericentric and paracentric) in recurrent abortions in patients referred to cytogenetics laboratory of Sarem Hospital

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Introduction: Recurrent abortion affects almost 15% of diagnosed pregnansis. More than 50% of miscarriages in the 1st trimester are due to chromosome abnormality. The aim of this study is to present the role of chromosomal inversions in recurrent miscarriages in patients referred to the Cytogenetics Laboratory of Sarem Hospital in Tehran. One group of inversions is pericentric inversion of chromosome 9, which is usually regarded as a normal population variant.

Materials and Methods: The samples were studied using high resolution GTG banding technique. For each patient, a minimum of 15 metaphases was examined by light microscopy.

Results: Pericentric inversion around centromere of chromosome 9 was observed in 29 patients (1.37%) and pericentric inversion in hetrochromatin region was found in chromosome 1 in one patient and chromosome Y in another patient, and one patient had pericentric inversion of chromosome 2. Chromosomal inversion involving other autosomal chromosomes included pericentric inversion of chromosomes 1, 5, 11 and 12 (1.9%), and paracentric inversion of chromosomes 3, 6, 7, 8, and 12 (0.23%).

Conclusion: The chromosomal imbalance of gametes may produce spontaneous abortions and malformed offsprings. This suggests that such inversions should not be ignored and they can play an important role in reproduction failure. However, we have shown that the rate of pericentric inversion of chromosome 9 is similar in different referral groups (1-2%) and similar to normal population and thus of no clinical significance.

Key words: *Pericentric and paracentric chromosome inversions, Recurrent abortions, inv (9).*

P-103
Kit Ligand (KL) promotes the primordial follicle growth in mouse vitrified ovaries

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Introduction: Following cryopreservation of ovarian tissue, the primordial follicles are well preserved with minimal damage. An alternative technique to improve the development of follicles within ovarian tissue is ovarian organ culture following cryopreservation. Kit ligand (KL) known as stem cell factor, steel factor or mast cell growth factor is involved in the activation of primordial follicles, oocyte growth and proliferation of granulosa and theca cells.
Materials and Methods: One week old mouse ovaries were collected and divided into vitrified and non-vitrified groups. Then they were cultured in the presence or absence of KL for 7 days. The development of ovarian follicles was evaluated by histology and also the mean area and hormonal level was analyzed during culture period. Apoptosis assessment was done using DNA laddering, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) method and caspase -3/7 activity assay.
Results: The proportion of preantral follicles and the level of hormones were increased in all cultured groups and it was significantly higher in KL treated groups than their control ($p<0.001$). DNA fragmentation and TUNEL positive signals were seen in vitrified cultured ovaries. The level of caspase -3/7 activity was higher in vitrified cultured ovaries.
Conclusion: KL could improve the development of follicles in vitrified cultured ovaries also it could act as anti-apoptotic factor during culture of vitrified samples. The development potential of follicles in vitrified groups was lower than fresh ovaries.
Key words: *In vitro culture, Kit Ligand, Vitrification, Caspase -3/7.*

P-104
Association between rs1264457 A/G polymorphism of *HLA-E* gene and recurrent pregnancy loss

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Introduction: Recurrent pregnancy loss (RPL) is defined as two or more consecutive miscarriages before twentieth week of pregnancy. The cause of 50% recurrent miscarriages is unexplained. The goal of this research was to investigate the association between RPL and rs1264457 A/G polymorphism of *HLA-E* gene.
Materials and Methods: In this case-control study we used Polymerase Chain Reaction Restriction Fragment Length Polymorphism (RFLP-PCR) to determine frequency of this polymorphism in 105 women with RPL in comparison with 109 healthy controls. The collected data were analyzed by the statistical package for the social sciences software (SPSS) and the chi-square test.
Results: The finding showed clear correlation between this polymorphism and RPL. The results will be presented in the congress.
Conclusion: If distinctive data increase frequency of this polymorphism in RPL, it can exponent influence of this polymorphism in generating RPL. We hope result of this investigation to be useful for finding some causes of unexplained recurrent pregnancy loss.
Key words: *Recurrent pregnancy loss, HLA-E, Polymorphism.*

P-105
Evaluation of the meiotic spindle and zona pellucida after vitrification of mouse MII oocytes

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Introduction: Oocyte vitrification is used as fertility preservation method for young aged females with cancer. The oocyte is one of the biggest mammalian cells with large volume of cytoplasm, compares with other cells. Also, oocyte has less permeability to cryoprotectants (CPAs), since high concentration of CPAs used in vitrification procedure with possible toxic effects. Evaluation of the effect of vitrificaiton on meiotic spindle (MS) and zona pellucida (ZP) of mouse MII oocytes using Polyscope technology was the subject of this study.
Materials and Methods: Ovulation induction performed for 6-8 weeks old NMRI mice by injection of 10 IU PMSG (IP) and 48 hr later with 10 IU HCG. After oviduct removal, COCs were denuded and MII oocytes were retrieved aseptically. The equilibration (7.5% EG, 7.5% DMSO) and vitrification (15% EG, 15% DMSO) solutions were prepared in Hams F10 supplemented with 20% HSA as base medium. For 3 steps of thawing, base medium plus 1, 0.5 and 0.25 M sucrose were used respectively. Assessment of ZP and MS performed at different time intervals after thawing.
Results: After warming, vitrified oocytes showed a significantly fast ZP digestion timing compared to the control group ($p<0.05$). Presence of MS was detected in 69.23% of vitrified oocytes 1 hr after warming, and presence of MS was showed in 11.11% of vitrified oocytes 2 hr after warming and 26.92% of vitrified-warmed oocytes showed sign of degeneration.
Conclusion: ZP digestion was faster in vitrified group rather than control and recovery of MS structure after vitrification/ warming at 37°C. Vitrification technique

still needs more modification before its application in fertility preservation program.

Key words: *Oocyte, Meiotic spindle, Zona pellucida, Vitrification.*

P-106

Protective effect of vitamin E on testicular germ cells and serum Malondialdehyde concentration in rats fallowing exposure to bisphenol A

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Introduction: Bisphenol-A (BPA), used in plastic industries, has toxic effects on the reproductive system. It can cause testis injury through the production of Reactive oxygen species (ROS).
Materials and Methods: 24 adult male rats (220±15 gr) were divided into 4 groups (n=6): control, BPA (250 mg/kg/day), Vit E (150 mg/kg/day) and BPA+ Vit E. All groups were orally treated for 56 days. By the end of the treatment, animals were killed, their right testis were taken out, fixed, sectioned, processed and stained with heidenhain azan method. The total number of germ and Sertoli cells was estimated using the optical dissector technique. Serum MDA levels were also measured with the spectrophotometric method. Data were statistically analyzed using one way ANOVA and Tukey's test and means were considered significantly different at $p<0.05$.
Results: A significant decrease in the total volume of testis, number of long and round spermatids, spermatocytes and Sertoli cells ($p<0.05$) and a significant increase ($p<0.03$) in the serum MDA level were found in rats treated by BPA compared to the control group. Histopathology observations revealed morphological changes in BPA-exposed rats including atrophy and vacuolation in the germinal epithelium. The above parameters were compensated to the control level in the BPA+ Vit E group.
Conclusion: Due to the antioxidant activity of vitamin E, it may ameliorate the damaging effects of bisphenol A on spermatogenesis.

Key words: *Bisphenol A, Vitamin E, Sertoli and germ cells number, Optical disector.*

P-107

Evaluation of follicular genes pattern and growth of preantral follicles after culture in alginate hydrogel following vitrification of the mouse ovarian tissue

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Introduction: This study was set up to evaluate the effect of ovarian tissue vitrification on the in vitro growth and pattern of follicular genes expression in mouse preantral follicles encapsulated within alginate hydrogel.
Materials and Methods: Ovaries of 12-14 days old female NMRI mice allocated into fresh control and vitrification groups. For cryopreservation, ovaries equilibrated with a solution (ES) that composed of 7.5% ethylene glycol (EG) and 7.5% dimethyl sulfoxide (DMSO) for 15 min and vitrification was performed by a solution (VS) that composed of 15% EG and 15% DMSO for 30 min then ovaries loaded to nitrogen with needle. Descending concentrations of sucrose (1, 0.5, 0.25 M) used for warming step. After histologic assessment of ovaries, in the next stage, pre-antral follicles was mechanically isolated from control and vitrified ovaries and was cultured for 12 days in 0.7% sodium alginate. Preantral follicles survival rate, growth, antrum formation and relative expression of oocyte- specific genes (*Bmp15*, *Gdf9*،*Fgf8*, *Igf1*, *Kit*, *Kit* ligand) was assessed after 1, 8 and 12 days of culture and finally maturation rate of oocytes was studied.
Results: Preantral follicles in vitrified group showed a lower survival rate on 8 and 12 days of culture ($p<0.05$) but could retain a comparable morphological appearance, growth and antral formation with the control group. Reduction of *Bmp15*, *Gdf9*, *Fgf8*, *Kit*, *Kit-l* showed during 12 days of culture ($p<0.05$). Although the expression of *Gdf9*, *Kit*, *Kit-l* in vitrification group was more than control group at 1st day of culture but all genes in both groups showed same expression after 12 days of culture ($p<0.05$).
Conclusion: Although vitrification of ovarian tissue reduces the survival rate, it is a safe method for preservation of preantral follicles and could not modify the relative expression of follicullar genes and oocytes maturation capacity.

Key words: *Follicular genes, Preantral follicle, Vitrification of ovarian tissue, Alginate.*

P-108

Effects of morphine on sperm parameters, protamine deficiency and DNA integrity in mice

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Introduction: Morphine as a natural alkaloid (opiate) is the most effective pain-relieving drugs and can be abused because of its high addictive potential. Opiate abuse is considered as one of the problems associated with poor semen production and sperm quality. Therefore, for the first time, this experimental study was carried out to evaluate the effect of intraperitoneal

injection of morphine on sperm parameters, protamine deficiency and DNA integrity of spermatozoa aspirated from cauda epididymis of mice.

Materials and Methods: Totally 24 adult male Balb/c mice (8 weeks old. 30g) were equally divided into 3 groups each containing 8 mice. Mice of group 1 served as control fed on basal diet, group 2 received basal diet and normal saline and group 3 received basal diet and morphine (15 mg/kg/daily, intraperitoneal) for 35 days. Finally right tail of epididymis of each mouse was cut and placed in Ham's F10 for 30 min. Released sperm were used to analyze count, motility, viability (eosin-nigrosin staining), morphology (Papanicolaou), protamine deficiency with chromomycin A3 (CMA3) and apoptosis via TUNEL assay.

Results: In morphine-treated mice a significant decrease was found in sperm viability, normal morphology, count and motility compared to other groups ($p<0.05$). In relation with protamine deficiency the rates of CMA3-reacted spermatozoa were similar in groups ($p>0.05$). In addition, in morphine-treated mice there was a significant increase in apoptosis compared to other groups ($p<0.05$).

Conclusion: The results showed that morphine abuse disturbs sperm parameters and DNA integrity but not protamine content of the sperm nucleus.

Key words: *Mice, Morphine, Sperm parameters, Apoptosis, Protamine deficiency.*

P-109

Study the protective role of jujube extract on growth index of mice fetuses exposed to Carbamazepine

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Introduction: Carbamazepine (CBZ) is an anticonvulsant drug that is widely used, primarily to treat seizures. One of the side effects of this medication during pregnancy is the reduced fetal growth. The jujube has been widely used in traditional medicine because of its special compound. The purpose of this study was to evaluate the preventive effects of aqueous extract of jujube on growth index of mice fetuses exposed to Carbamazepine.

Materials and Methods: In this study, 100 Balb/C mice (28-30 gr; 8-9 weeks-old) were divided into eight experimental and two control groups. Experimental groups (I, III, V) and (II, IV, VI) received daily intraperitoneal injections of 50 and 100 mg/kg of CBZ, respectively. Experimental groups (III, IV) and groups (V, VI) were gavaged doses of 200 and 400 mg/kg of aqueous extract of jujube respectively. Groups VII and VIII received only doses of 200 and 400 mg/kg of aqueous extract of jujube respectively. Control groups I and II were gavaged to normal saline and tween respectively. Injections were done on 0-15 gestational days (GD), and aqueous extract was gavaged along with CBZ starting 10 days prior to gestation. On GD 18, fetuses were removed for weighing and crown-rump measuring. Data were analyzed by ANOVA and Tukey tests using SPSS (version 18) at 0.05%.

Results: Average weight and body length of fetuses in experimental groups I and II, that received CBZ, significantly reduced compared with control groups. In the fetuses of experimental groups V (1.20±0.38) and VI (1.21±0.21 gr) that recieved jujube extract (400 mg/day) with CBZ, the mean weight of these fetuses increased meaningfully in comparison with the experimental groups I (0.93±0.19 gr) and II (0.92±0.26 gr) respectively.

Conclusion: This study showed that aqueous extracts of jujube can have preventive effects on reducing effect of CBZ on birth fetal weight.

Key words: *Growth index, Jujube, Carbamazepine, Mice.*

P-110

Effectiveness of mindfulness-based cognitive therapy on improvement of perceived stress and infertility in infertile women undergoing IVF irrational cognitions

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Introduction: Effectiveness of mindfulness-based cognitive therapy on improvement of perceived stress and infertility in infertile women undergoing IVF irrational cognitions.

Materials and Methods: This clinical trial with pre-test post-test was done in 1331 infertile women referred to the Reproductive Health Research Center, Crescent Hospital, UAE who were treated with IVF. In total, 24 infertile women filled an irrational cognitions questionnaire related to childbearing and infertility. They were divided into experimental and control groups. The experimental group received mindfulness-based cognitive therapy for 8 sessions of 2 hr each. The control group did not receive any mental health services. Those two questionnaires were completed before and after the intervention. Data were analyzed by SPSS and $p<0.05$ was considered significant.

Results: There were no significant differences between the experimental and control groups in terms of reduction of perceived stress of infertility. But, the improvements in the recognition of irrational for childbearing between two groups was significantly different.

Conclusion: Teaching mindfulness-based cognitive therapy on improvement of perceived stress and irrational cognitions related to fertility in infertile women undergoing IVF treatment is effective.

Key words: *Perceived stress, Infertility, IVF treatment, Mindfulness-based cognitive therapy, Irrational cognitions of parents.*

P-111
Genotype and phenotype frequencies of paraoxonase 1 in fertile and infertile men

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Introduction: Paraoxonase1 (PON1) is a glycoprotein associated with high density lipoprotein and has antioxidant activity. The impact of PON1 in various stages of spermatogenesis has also been suggested.
Materials and Methods: Q192R variants of *PON1* were determined in 150 fertile and 150 infertile men using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Plasma arylesterase and paraoxonase activities were detected by spectrophotometry and malondialdehyde (MDA) level was measured using thiobarbituric acid.
Results: Our results showed no significant difference in the distribution of *PON1* genotypes and alleles between fertile and infertile groups. However morphology and motility of sperm were associated with various genotypes of *PON1*. The number of fertile males with the BB phenotype (high activity) was significantly higher than that of infertile males, whereas the number of individuals with the AB phenotype (moderate activity) was statistically higher in infertile men compared with the fertile group. Additionally, MDA and arylesterase activity levels were significantly higher in infertile subjects compared with fertile men.
Conclusion: We speculate that the low activity of PON1 can be a risk factor for male infertility probably due to a decrease in antioxidant activity of PON1 and increase in lipid peroxidation.
Key words: *Genetic polymorphism, Male infertility, Paraoxonase 1, Reproduction.*

P-112
Saccharin consumption increases sperm DNA fragmentation and apoptosis in mice

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Introduction: Saccharin is an artificial non-caloric sweetener that used to sweeten products such as drinks, candies, medicines, and toothpaste, but our bodies cannot metabolize it. Sodium saccharin is considered as an important factor in tumor promotion in male rats but not in humans.
Materials and Methods: Totally 14 adult male mice were divided into 2 groups. Group 1 served as control fed on basal diet and group 2 or experimental animals received distilled water containing saccharin (0.2% w/v) for 35 days. After that, the left cauda epididymis of each mouse was cut and placed in Ham's F10. Swimmed-out spermatozoa were used to analyze count, motility, morphology (Pap-staining) and viability (eosin-Y staining). Sperm DNA integrity, as an indicator of apoptosis, was assessed by sperm chromatin dispersion (SCD) and terminal deoxynucleotidyl transferase (TUNEL) assay.
Results: Following saccharin consumption, we had a reduction in sperm motility with respect to control animals (p=0.000). In addition, the sperm count diminished (17.70±1.11 in controls vs. 12.80±2.79 in case group, p=0.003) and the rate of sperm normal morphology decreased from 77.00±6.40 in control animals into 63.85±6.81 in saccharin-treated mice (p=0.001). Also, we saw a statistically significant increase in rates of sperm DNA damage and apoptosis in experimental group when compared to control one (p=0.001, p=0.002 respectively).
Conclusion: Saccharin consumption may have negative effects on sperm parameters, and increases the rate of sperm DNA fragmentation and apoptosis in mice.
Key words: *Sperm, Saccharin, Apoptosis, Mice.*

P-113
Increased telomeric repeat containing RNA (TERRA) levels in cumulus cells of infertile polycystic ovary syndrome patients

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Introduction: One of the important reasons of anovulation based infertility is polycystic ovary syndrome (PCOS) which occurs in 5-10% of women in reproductive age. It seems that the length of telomere, TTAGGG tandem repeats, is related to proliferation and differentiation events during follicular development including the mechanisms which regulate successful reproduction. It is widely recognized that bi-directional communications exist between oocytes and the surrounding cumulus cells which are essential for the production of competent oocytes. During the past few years, study on cumulus cells showed that the length of telomere is longer in cumulus cells of mature oocytes than immature ones. Until now, telomeres have been considered to be transcriptionally silent but recent

studies have clearly shown that transcription of subtelomere regions produces telomeric repeat containing RNA, named TERRA. In this study, we tried to identify the correlation of TERRA levels with oocytes maturation by evaluating of TERRA transcripts in cumulus cells of PCOS patients.

Materials and Methods: For this respect, cumulus cells were collected from 6 PCOS patients and 9 healthy women with male factor infertility through ICSI/ IVF procedure. TERRA transcripts were measured by using quantitative real- time PCR.

Results: Our data showed that the level of TERRA transcripts was increased significantly in PCOS group vs. control group ($p<0.05$).

Conclusion: This finding implies a considerable association between TERRA transcript levels and PCOS, so it can be concluded that any changes in levels of TERRA transcripts can be judged as a potential marker for the quality of oocytes in ART procedure.

Key words: *TERRA, Telomere, PCOS, Cumulus cells.*

P-114
Epigenetic modification profile of endometrium in endometriosis patients

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Introduction: Endometriosis defined as the presence of endometrium-like tissue outside of the uterine cavity, is a common gynecologic disorder. Although endometriosis is a multifactorial disease and the exact etiology is not clearly understood, recently, some evidence suggests that epigenetic is associated with the molecular features of endometriosis. Two main epigenetic regulatory mechanisms, DNA methylation and histone modifications regulate expression of genes and recognize the states of diseases. The aim of this study was to investigate alterations of DNA methylation and histone acetylation and methylation levels in eutopic and ectopic endometrium of endometriosis patients.

Materials and Methods: Informed consents were gained from all patients then eutopic and ectopic endometrium samples (n=5) were collected from endometriosis patients undergoing surgery and biopsy, as well as endometrial tissues from healthy fertile women (n=5) during the proliferative phase of menstrual cycle. Chromatin extracts from samples were prepared following fixation and then shearing into fragments by sonication. Nucleosome ELISA was performed on chromatin extracts, in order to identify global histone H3K9 acetylation/ methylation and DNA methylation, using antibodies against H3K9ac, H3K9me and MeCP2, respectively.

Results: We have identified global histone H3K9 hypermethylation in ectopic and eutopic endometrium, compared with controls. A significant hyperacetylation at histone H3K9 was observed in eutopic samples compared to ectopic and control groups. Furthermore, eutopic endometrial samples were globally DNA hypermethylated in comparison with controls.

Conclusion: These results clearly show an epigenetic switch in endometrial and endometriotic tissue of patients with endometriosis, in the way that aberrant DNA methylation and histone acetylation/ methylation status may play a dynamic role in occurrence of endometriosis and support the opinion that epigenetic abnormalities have causative functions in endometriosis.

Key words: *Endometriosis, Endometrium, Epigenetic, Methylation, Acetylation.*

P-115
The effects of chronic and acute ethanol administration on sperm chromatin parameters in mice

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Introduction: Sperm chromatin can be damaged by effects of some toxic materials. Among this, ethanol is one of the reproductive toxins that consumes abuse in men and may be associated with poor sperm quality. The purpose of this investigation was to examine the acute and chronic effects of ethanol consumption on sperm parameters and chromatin assay through testing the effects in mice.

Materials and Methods: Animals were randomly divided into 3 groups consisting of 10 mice each. Group 1 and 2 served as alcohol group and were received a daily dose of (3 g/kg body weight as 25%, v/v) ethanol by I.P. for four and eight weeks respectively. Group 3 (control group) was given normal access of food and water. The subdivisions and dosages were based on past works. At the end of treatments, laparotomy was conducted to expose the reproductive system, cauda of epididymis were immediately dissected out for evaluations of sperm parameters and various tests were used to analysis of the chromatin integrity of sperm.

Results: Various tests such as aniline blue (AB), chromomycin A3 (CMA3), toluidine blue (TB), and acridine orange (AO) were used to analysis the sperm quality. We did not find any statistically significant differences in acute and control groups. Although sperm count and motility were increased but viability, normal morphology, DNA and chromatin integrity were unaffected by ethanol in acute group and were identical with control group. Our results demonstrated that count,

motility and viability of sperms were dramatically decreased in chronic treated animals. Besides of that, DNA and chromatin integrity were significantly decreased.
Conclusion: Therefore, ethanol abuse can induce abnormality in sperm structure and function, and this may be one possible cause of male infertility.
Key words: *Sperm, Ethanol, Chromatin integrity.*

P-116
Comparison of oral dydrogesterone with vaginal progesteronefor luteal support in IUI cycles: A randomized clinical trial (RCT)

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Introduction: Progesterone supplementation is the first line of treatment in ovarian stimulation. This study was conduct to compare the effect of oral dydrogestrone with vaginal cyclogest on luteal phase support in intrauterine insemination (IUI) cycles.
Material and methods: This prospective, randomized, double blind study was performed in a local infertility center from May 2013-2014. It consisted of 150 infertile women younger than 35 years old undergoing ovarian stimulation for IUI cycles. They underwent ovarian stimulation with oral dydrogesterone (20 mg) as group A and vaginal cyclogest (400 mg) as group B in preparation for the IUI cycle. Clinical pregnancy and abortion rates, mid luteal progesterone (7 days after IUI) and patients' satisfaction were compared between the two groups.
Results: The mean serum progesterone levels was significantly higher in group A compared to group B (p=0.001). Pregnancy rate in group A was not statistically different from group B (p=0.58). Abortion rate in group B was higher than this rate in group A, but the difference was not statistically significant (p=0.056). Satisfaction rates were significantly higher in group A compare to group B (p<0.001).
Conclusion: We concluded that oral dydrogestrone is as effective as vaginal progesterone for luteal-phase support in woman undergoing IUI cycles. Moreover, the mean serum progesterone levels and satisfaction rates in dydrogestrone group were higher than cyclogest group.
Key words: *Dydrogesterone, Cyclogest, Luteal-phase, Infertility.*

P-117
The detrimental effects of alcohol on sperm parameters and DNA integrity in diabetic mice

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Introduction: Diabetes mellitus (DM) is a chronic disease that can affect male reproductive function at multiple levels. Ethanol can suppress reproductive function and sexual behavior in laboratory animals and humans. Alcohol also is considered as one of the problems associated with poor semen production and sperm quality. The main goal of this study is to examine the effect of alcohol on sperm parameters and DNA integrity in diabetic mice.
Materials and Methods: Totally 32 adult male Syrian mice (10 weeks old, 35 gr) were divided into 4 groups, mice of group 1 served as control fed on basal diet, group 2 received streptozotocin (STZ) (200 mg/kg, single dose, intra peritoneal) and basal diet, group 3 received alcohol (10 mg/kg, water-soluble) and basal diet and group 4 received streptozotocin and alcohol. After 35 days, the cauda epididymis of each mouse was dissected and placed in 1 mL of pre-warm Ham's F10 culture medium for 30 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability. Also the sperm chromatin quality and DNA integrity, was evaluated with Aniline blue (AB), Toluidine blue (TB), Acridine orange (AO) and Chromomycin A3 (CMA3) staining.
Results: In this study all of the sperm parameters were significantly diffrent between groups (p=0.001). Also, regarding the sperm DNA integrity tests, the results from four tests showed significant differences between groups. Also, in diabetes+ alcohol mice, a significant increase was found in mean tests compare to other groups.
Conclusion: Although the DM may have bad effects on sperm fertility potential and DNA integrity, but, on the other hands, consumption of alcohol in diabetic mice, may have detrimental effects on sperm parameters, sperm function and also sperm chromatin condensation in experimentally-induced diabetic mice.
Key words: *Mice, Sperm parameters, DNA integrity, Diabetes, Alcohol.*

P-118
Protective effect of Silymarin on plasma membrane integrity, acrosome integrity and mitochondrial membrane potential in cadmium-treated mice

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Introduction: Cadmium as a heavy metal and environmental pollutant is able to exert numerous

undesirable effects on human reproduction by inducing oxidative stress. Silymarin, a polyphenolic flavonoid extracted from the seeds of Silybum marianum, has effective antioxidant properties.

Materials and Methods: Adult NMRI mice were divided into four groups: 1) control 2) cadmium chloride (5 mg/kg, sc) 3) Silymarin (100 mg/kg, ip) and 4) Silymarin + cadmium. Treatment period was 24 hours. After treatment mice were dissected and their epididymis was cut into small pieces in HTF medium in order to swim out spermatozoa. The spermatozoa from different groups were used to evaluate sperm parameters. Sperm plasma membrane integrity, acrosome integrity and mitochondrial membrane potential assessed by Hoechst and propidium iodide, coomassie blue and rhodamin staining respectively. Data were analyzed with one way ANOVA and $p<0.05$ was considered significant.

Results: In cadmium-treated mice, the percentage of intact membrane and acrosome were significantly decreased compared to the control. In addition, mitochondrial membrane potential was significantly decreased in cadmium-treated mice in comparison with the control. In Silymarin + cadmium group, Silymarin could significantly compensate the adverse effects of cadmium compared to cadmium group.

Conclusion: The present study showed that cadmium induces toxic effects on mice sperm plasma membrane integrity, acrosome integrity and mitochondrial membrane potential and Silymarin can compensate the toxic effects of cadmium on these sperm parameters.

Key words: *Cadmium, Silymarin, Sperm parameters.*

P-119

Evaluating quality of well-being, marital adjustment and sexual dysfunction between infertile women

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Introduction: Infertility is a stressful experience and has a high impact on the infertile women's psychological status. Infertility and it is treatment create a major and prolonged crisis for the women and create a heavy psychological trauma for the women. The effectiveness of infertility treatment depends on success rate of the treatment facility, the emotional well-being and sexual health of the women seeking treatment.

Materials and Methods: For the propose of the study, 50 fertile and 50 infertile women were selected randomly (n=100) from Midwife IVF and Fertility Research Hospital in Mysore-India. All subjects were assessed using Quality of Well-Being Scale-Self Administered (QWB-SA), Marital Adjustment Test (MAT) and Massachusetts General Hospital Sexual Functioning Questionnaire (MGH) questionnaire were administered.

Results: The results showed significant difference between fertile and infertile women assessment on Quality of Well-Being and Marital Adjustment score. Compare with the fertile women, the patients with infertility had significantly lower scores in the sexual interest and sexual arousal domains and lower frequency of intercourse. The patients with infertility retrospectively reported an orgasm, lubrication and sexual satisfaction score that was similar to that of the fertile women.

Conclusion: Women with a diagnosis of infertility were found to be at higher risk for sexual dysfunction when compare with fertile women. Further infertile women reported poor marital adjustment and quality of well being compare with controls. The results suggest to apply counseling and psychotherapy services in the infertility centers to reduce the psychological problems, increase Quality of Well-Being, Marital Adjustment and identify the factors that can contribute to the development of sexual dysfunction on women facing infertility to help them to enhance fertility chances.

Key words: *Quality of well-being, Marital adjustment, Sexual dysfunction, Infertile women.*

P-120

Assessment of aerobic bacterial and fungal contaminations in liquid nitrogen tanks

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Introduction: Cryopreservation technologies play an important role in assisted reproduction for both medical and research purposes. But, there are some concerns about the technical and biological safety of this technology because of pathogen transmission during cryopreservation and germplasm banking. The aim of this study was to evaluate the presence of microbial contaminations in liquid nitrogen (LN2) and carrier tanks used in different laboratories of our reproductive science institute.

Materials and Methods: In this descriptive study, different parts of 20 carrier and storage LN2 tanks, such as LN2, vapors, cryocans, and inner surface were qualitatively evaluated for microorganism contaminations through the assessment of bacterial and fungal growth in minimal and selective Petri dishes.

Results: Two fungal (Candida albicans and aspergillus flavus) and two aerobic bacterial microorganisms (Ecoli, Pseudomonas aeruginosa) were the most

common findings in bottoms, vapors, and inner surfaces of 2 carriers and 11 storage LN2 tanks. There were Pseudomonas aeruginosa growths in 8 cryocans from contaminated tanks. Also, there were staphylococcus aureus and acinetobacter baumannii contaminations in one storage tank.

Conclusion: Theoretically, LN2 tanks can be considered as a possible source of pathogen transmission. Therefore, performing quality control assays, like microorganism detection tests for LN2 and supplementary equipments should be considered as a routine practice in IVF clinics. Sterilization of tanks should be performed on regular basis.

Key words: *Bacterial growth, Contamination, Liquid nitrogen.*

P-121

Effect of non-polar extract of Phaleria macrocarpa on sperm quality in adult rats

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Introduction: Diminish production of testosterone hormone could affect sperm quality; hence contribute to the infertility in man.

Materials and Methods: 25 Sprague Dawley adult male rats were randomly divided into five groups. Rats were supplemented orally once a day with 0.25 ml Tween 20 solution containing non-polar hexane extract of PM at one of the following concentrations: 0, 6, 12, and 60 mg/kg for seven weeks. Commercial testosterone hormone was used as positive control. On the last day of supplementation period, all rats were sacrificed and orchidectomy technique was performed to collect semen sample from cauda epididymis of the testis. The sperm count, motility, viability and morphology were determined.

Results: The results showed that the sperm motility was significantly improved by non-polar PM extract ($p<0.05$) with the value of 66.98% (6 mg/kg), 63.96% (12 mg/kg) and 65.21% (60 mg/kg) compared to 44.08% for 0 mg/kg group. The supplementation of PM extract however did not improved the sperm count, sperm viability and sperm morphology. The sperm count was 852 (0 mg/kg), 918 (6 mg/kg), 906 (12 mg/kg), 1030 (60 mg/kg) and 1027 million cells/ml (commercial testosterone). The sperm viability was 85.90 (0 mg/kg), 85.26 (6 mg/kg), 85.45 (12 mg/kg), 87.88 (60 mg/kg), and 89.32% (commercial testosterone). The sperm morphology was 62.5% (0 mg/kg), 63.7% (6 mg/kg), 72.2% (12 mg/kg), 67.0% (60 mg/kg) and 64.5% (commercial testosterone). The results showed that non-polar PM extract only improved the sperm motility and did not have any effect on the sperm count, morphology and viability.

Conclusion: The study concludes that non-polar extract of PM has no compound that can improve sperm quality except on the motility of the sperm.

Key words: *Phaleria macrocarpa, Sperm count, Sperm motility, Sperm viability, Sperm morphology.*

P-122

Protective effect of ascorbic acid on testicular tissue in mice exposed with sodium arsenite

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Introduction: Arsenic is one of the major environmental contaminants with carcinogenic and toxic effects. Ascorbic acid as an antioxidant is able to restrict oxidative stress. The aim of this study was to investigate the adverse effect of sodium arsenite on the mice testicular tissue as well as to examine whether vitamin c is able to ameliorate this effect.

Materials and Methods: 24 adult male NMRI mice were randomly allocated into four groups (n=6) including control, sodium arsenite (7 mg/kg/day, orally), ascorbic acid (150 mg/kg/day, orally) and finally sodium arsenite+ ascorbic acid. Mice were treated for 35 days. At the end, mice were sacrificed and their right testis were taken out, fixed, processed and stained with Heidenhain azan method. The total volume of testis, volume of interstitial tissue, volume of seminiferous tubules, diameter and of length of seminiferous tubules, germinal epithelium height and basement membrane thickness were estimated using stereological methods. Data were analyzed using one way ANOVA and Tukey's test and means difference were considered significant at $p<0.05$.

Results: A significant reduction in total volume of testis, volume of seminiferous tubules and its diameter and germinal epithelium height, was found in sodium arsenite group compared to control group ($p<0.04$). In ascorbic acid + sodium arsenite group, the above parameters were significantly increased compared with sodium arsenite group ($p<0.02$). In addition, germinal epithelium height and diameter of seminiferous tubules were increased in mice treated with ascorbic acid alone compared to the control group ($p<0.002$).

Conclusion: The results indicate that ascorbic acid may be useful in reducing the sodium arsenite- induced toxic effects on testicular tissue.

Key words: *Sodium arsenite, Ascorbic acid, Stereology, Testis.*

P-123

The comparison of anxiety and depression rate between medical staff of Infertility Centers and Obstetrics and Gynecology Centers of Yazd, Shiraz, Isfahan and Kerman Hospitals

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Introduction: Mental health is an important issue in personal, social and occupational function of anyone in the life; moreover, mental problems have many negative consequences on the quality of professional work. Regarding the close and continuous interaction of infertility staff with hopeless infertile couples and in the contrary the atmosphere of happiness especially in Obstetric Wards it make sense that anxiety and depression rates would be different between them.

Materials and Methods: This study was a descriptive-correlation study based on cross-sectional method. 199 individuals who were the staff of Infertility Centers and Obstetrics and Gynecology Wards in four provinces enrolled in this study through stratified sampling. Data collection was done by demographic questionnaire, Spiel Berger and Beck depression inventory tests. Data were analyzed by SPSS software and ANOVA test.

Results: The results showed that there is statistically significant higher rate of anxiety in staff of Obstetrics and Gynecology Wards in Isfahan (54.69±13.58) while there was a higher rate of depression in staff of Infertility Center in Shiraz (14.94±10.87). Overall, correlation between anxiety, depression and work place was statistically significant (p=0.047 and 0.008 respectively). According to ANOVA test, the mean value of anxiety level was higher in the staff of Obstetrics and Gynecology Centers of Isfahan, Shiraz, Shahid Sadoughi and Madar Hospitals in Yazd (p=0.03, 0.006, 0.008, 0.012 respectively) and Infertility Center of Yazd (p=0.00).

Conclusion: Comparison of the mean value of anxiety in staff of Obstetrics and Gynecology Wards and Infertility Centers was not significant in these four centers except for Isfahan Center. As long as we know that infertile couples have little chance for success rate and Obstetrics and Gynecology patients have little risk of failure in treatment it could be mentioned that the anxiety and depression in the staff are not correlated with the client illness.

Key words: *Anxiety, Depression, Infertility centers, Obstetrics and gynecology centers.*

P-124

Developmental competence of immature oocytes aspirated from ovarian antral follicles as a method for fertility preservation

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Introduction: Advances in the treatment of cancerous women in reproductive age have markedly been increased recently. Also, in vitro maturation (IVM) of immature oocytes collected from ovary has been proposed for fertility preservation. In addition, the morphology of oocytes post IVM is one of the factors determining its developmental competence. By using the non-invasive PolScope system, the meiotic spindle and zona pellucida (ZP) can be assessed in living oocytes.

Materials and Methods: The ovarian cortex from 26 cancer patients (21-45 years old), were obtained directly from collaborating hospitals, and transported to the IVF center on ice. 61 immature oocytes were aspirated, of which 18 (29.5%) were degenerated and discarded. The remaining 43 (70.5%) healthy oocytes were cultured for 48 hr IVM culture media. The rate of maturity was assessed and the ZP birefringence and meiosis spindle (MS) were imaged with Polscope technology.

Results: 43 immature oocytes underwent IVM technology, of which 30.2% reached viable metaphase II (MII) oocytes. The ovarian tissues of 9 (34.6%) women were lacking oocytes at any stage. There was a positive correlation between the recovered number of oocytes and the ovarian volume. During polarized light microscopy examination, MS could be visualized only in one of the MII oocytes, but high ZP birefringences were observed in most of the oocytes post IVM (61.5%).

Conclusion: Oocytes maturation post IVM from unstimulated ovaries showed a good developmental competence in cancerous patients. Further studies should be performed to advance the oocyte maturation program, such as co-culture system, for fertility preservation.

Key words: *Fertility preservation, Ovarian tissue, IVM, Polscope.*

P-125

Effect of Heracleum persicumm oil and alcoholic extracts on sperm parameters and chromatin quality in mice

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Introduction: Seminal plasma from infertile men has lower antioxidant levels than that of fertile men, particularly of patients whose semen have poor sperm motility. Evaluation of the importance and the effects of plant derived drugs on fertility of laboratory animals have long been recognized. Antioxidant activity has been reported from Heracleum persicum (Golpar).
Materials and Methods: 80 adult male mice (10 weeks old, 35 gr) were divided to 3 groups: group1 received hydroalcoholic extract (1000 mg/kg, ip), group 2 received oil extract (200 ml/kg, ip) and group 3 serving as the sham control group that received water. After 35 days, the cauda epididymis of each mouse was dissected and placed in 1 mL of pre-warm Ham's F10 culture medium for 30 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability. The sperm chromatin quality and DNA integrity, was evaluated with Aniline blue (AB), Toluidine blue (TB), Acridine orange (AO) and Chromomycin A3 (CMA3) staining.
Results: In sperm analysis, progressive and non-progressive motility were significantly differences between groups. Regard to sperm chromatin quality, the results of TB and AO tests showed statically significant differences between groups, but in AB and CMA3 staining, we didn't see any differences between them. So we can say that the Heracleum persicum extracts doesn't have any detrimental effects on histone-protamines replacement during the testicular phase of sperm chromatin packaging.
Conclusion: According to our results, Heracleum persicum extracts as an antioxidant although improved sperm motility but may influence the male fertility potential via affecting DNA/ chromatin quality in mice.
Key words: *Heracleum persicum extracts, Sperm, Mice, Chromatin.*

P-126
Effect of vitrification on high magnification morphology, chromatin condensation, and fertility potential of human spermatozoa

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Introduction: Sperm vitrification is a technique of ice- and CPA- free cryopreservation by direct plunging of a sperm suspension into liquid nitrogen (LN). Sperm characteristics of motility and morphology are important for normal spermatozoa-oocyte interaction. However, defects on chromatin condensation can cause vacuolization on sperm morphology. Motile sperm organelle morphology examination (MSOME) is an unstained real time high magnification that analysis viable sperm morphology. This study investigated the influence of vitrification on human sperm structure by MSOME technique, and fertility potential by zona binding assay (ZBA) and chromatin condensation by toluidine blue (TB) and aniline blue (AB) assessment.
Materials and Methods: 30 normozoospermic ejaculates were prepared by swim up technique, and supernatants were divided into two parts: fresh and vitrified groups. For vitrification, sperm suspension was mixed with equal volume (1:1) of Hams F10 +5% HSA +0.5 M sucrose. Then, 30 µl sperm suspension was dropped into LN. Warming was performed by quick submerging spheres into pre-warmed 5ml Hams F10 with 5% HSA at 37˚C. Sperm motility, stained morphology, MSOME and ZBA were evaluated for each sample. Three classes were considered for MSOME analysis: high quality sperm with a score of 4-6 (Class 1); medium-quality sperm with a score of 1-3 (Class 2); low-quality sperm with a score of 0 (Class 3). 2×10^6 spermatozoa in each 25µl droplet containing 4 Oocytes were performed for ZBA. In addition, samples were fixed for TB and AB assessments.
Results: Cryopreservation significantly reduced both progressive motility and normal morphology of spermatozoa. There was no significant differences between the rates of MSOME in class 1 (14.93±14.66 vs. 13.56±11.34), class 2 (53.53±13.99 vs. 55.63±12.16), class 3 (31±20.81 vs. 30.80±16.03) pre and post vitrification, respectively. However, vitrification reduced the fertility potential of spermatozoa from normozoospermic samples (13.40±22.73 vs. 9.00±13.87) and chromatin condensation (TB: 60.32±16.60 vs. 57.28±17.19) (AB: 38.32±8.14 vs. 34.35±6.87).
Conclusion: Vitrification had adverse effects on sperm parameters of motility and morphology. However, this technique did not increase the rate of vacuolization of sperm head or severe alteration in fertility potential. Since, the majority of human spermatozoa contained vacuolization in head region, it is highly recommended to use MSOME technology for assessment of sperm fine morphology during clinical microinjection procedure.
Key words: *Sperm vitrification, Motile sperm organelle morphology examination (MSOME), Zona binding assay (ZBA).*

P-127
Vitamin C attenuates detrimental effects of diabetes mellitus on sperm parameters, chromatin quality and rate of apoptosis in mice

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Introduction: Diabetes Mellitus (DM) may affect male reproductive functions at multiple levels. It is shown that intake of antioxidants such as vitamin C and E can reinforce the stability of testicular blood barrier and

protect sperm DNA from oxidative stress.The main goal was to examine the protective effects of vitamin C on sperm parameters, sperm chromatin condensation and apoptosis in experimentally-induced diabetic mice.

Materials and Methods: 28 adult Syrian mice were divided into 4 groups. In group 1, the mice were diabetic that received a single dose of Streptozocin (STZ) (200 mg/kg) intra-peritoneally (ip). Group 2 included diabetic mice that received vitamin C (10 mg/kg/daily, ip). Mice in group 3 received vitamin C and group 4 was considered as control. After 35 days, sperm analysis was done accordingly. To asses sperm chromatin and DNA quality, we used aniline blue (AB), toluidine blue (TB), chromomycin A3 (CMA3), acridine orange (AO) and terminal transferase mediated deoxyuridine triphosphate biotin end labeling (TUNEL) tests.

Results: All of the sperm parameters (count, motility, morphology and viability) had significant reduction in diabetic mice but, the data showed a significant increase in all of the sperm parameters in diabetic + vitamin C when compared with diabetic and control animals ($p<0.05$). There were significant differences ($p<0.001$) between groups regarding TB staining (48.8±5.92 vs. 34.3±4.13), AO test (35.9±6.11 vs. 20.8±2.89) and TUNEL test (39.42±7.18 vs. 22.00±3.65) in diabetic and diabetic + vitamin C groups, respectively. Nevertheless, in CMA3 and AB staining assays, there were not any significant differences between different groups.

Conclusion: Vitamin C, as a potent antioxidant, can attenuate detrimental effects of diabetes mellitus on the sperm parameters, chromatin quality and apoptosis in an experimental model.

Key words: *Sperm, Diabetes mellitus, Mice, Vitamin C, Chromatin, Apoptosis.*

P-128

DNA fragmentation in mammalian spermatozoa and its relationship with male infertility

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Introduction: It has been suggested that altered nuclear chromatin structure or damaged DNA in spermatozoa is implicated as a possible cause of increased infertility in males. Since the first reports on sperm DNA integrity, this subject has become the focus of numerous studies. Up to 8% of infertile men have been shown to have high levels of sperm DNA fragmentation despite a normal semen analysis. Currently, there are eight major tests of sperm DNA fragmentation, including the comet assay, sperm chromatin structure assay (SCSA), acridine orange test (AOT), tritium-labeled 3H-actinomycin D (3H-AMD) incorporation assay, terminal TdT-mediated dUTP-nick-end labeling (TUNEL) assay, in-situ nick translation (ISNT) assay, DNA breakage detection-fluorescence in-situ hybridizations (DBD-FISH) assay and sperm chromatin dispersion (SCD) test. New studies suggest that sperm with certain levels of DNA fragmentation serve as a strong predictor of reduced male fertility. Diagnosis of the fertilizing ability of a semen sample is important for consistently high reproductive efficiency. This paper aimed at investigating the potential use of sperm DNA fragmentation (SDF) to improve the routine screening of male infertility.

Materials and Methods: This article discusses some of the current techniques employed for evaluating chromatin structure or DNA damage in spermatozoa. A review of recent bibliography was carried out in PubMed, Google scholar and SID by the use of relevant keywords, in order to evaluate the possible correlation between the conventional seminal parameters and sperm DNA fragmentation assessment as diagnostic tools in male infertility evaluation.

Results: A negative correlation was found between sperm characteristics and the proportion of sperm showing DNA fragmentation. For fragmentation >30%, a significant decrease of the fertilization rate was observed.

Conclusion: The proportion of sperm with DNA fragmentation appears to be potentially useful as a predictor of infertility. Sperm DNA fragmentation is a parameter worth integrating in routine clinical practice. However, additional large scale studies based on the optimization of sperm DNA integrity are needed.

Keywords*: Spermatozoa, Male, DNA damage, Infertility.*

P-129

Establishment of spermatogonial stem cell line in mouse testicular culture

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Introduction: In the seminiferous tubules of the testis spermatogonial stem cells (SSCs), Sertoli cells and differentiating cells during spermatogonesis are present.

Materials and Methods: Here we report the establishment of a new VASA/ DAZL/ PLZF/-, Sox9/ Nanog/ GFRa1 low and KLF4, SOX2, VIMENTIN, N-MYC, OCT4+ cell line both from neonate and old mice, which could be expanded on SNL feeders for more than a year.

Results: Unlike undifferentiated SSCs they have a smaller nuclear/cytoplasm ratio in EM. FACS analysis

showed the expression of CD49, CD29, CD9, GFRa1 and E-CAD. In Fluidigm analysis the cells expressed pluripotency markers OCT4, NANOG, SOX2 and KLF4 but only partially expressed the typical germ cell profile of SSCs. One month after transplantation in busulphan treated NOD SCID mice we observed localization of GFP labelled cells in the basal compartment of the seminiferous tubule.

Conclusion: These more differentiated SSCs could provide an ideal cell system for studying both pluripotency and in vitro differentiation of SSCs to sperm and also provide a new strategy for isolation of SSCs from neonate and old mice by morphology based selection.

Key words: *Germ cells, Spermatogonial stem cells, Testicular culture.*

P-130

The association between TNF-alpha -308 polymorphism and susceptibility to spermatogenic failure

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Introduction: Approximately half of infertility reasons are related to male factors among which genetic etiology is the main cause. Signaling molecules can regulate spermatogenesis during regulation of germ cells maturation. Tumor necrosis factor-α (TNF-α) is one of the prominent cytokines that supports signaling molecules and it can affect spermatogenesis.

Materials and Methods: This case-control study includes 50 azoospermic men who referred to Yazd Research and Clinical Center for Infertility and 50 healthy controls. After sperm analysis, DNA was extracted and Restriction Fragment Length Polymorphic-Polymerase Chain Reaction (RFLP-PCR) was carried out for TNF alpha -308 polymorphism.

Results: The frequencies of A allele and G allele were 58% and 42% in azoospermic group, and 34% and 67% among controls, respectively. Among azoospermic patients, 42% presented AA homozygous genotype, 32% AG heterozygous genotype, and 26% GG homozygous genotype. In the control group, 20% presented AA homozygous genotype, 28% AG heterozygous genotype, and 52% GG homozygous genotype. According to our findings, A allele (p=0.001, OR=2.681) and AA genotype (p=0.030, OR=2.89) have a positive association with spermatogenic failure in the cases.

Conclusion: Our data suggest that the polymorphism might be associated with the risk of spernatogenic failure in Iranian azoospermic and it can be used as an infertility marker for screening as well as further treatment.

Key words: *Azoospermic, Cytokines, TNF-α, Polymorphism, RFLP-PCR.*

P-131

The role of FSH receptor gene alterations in poor ovarian response to gonadotropins

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Introduction: Poor ovarian response is an infertility disorder in which women's ovaries don't have proper response to gonadotropins. Follicle stimulating hormone (FSH) has a critical role in the maturation of the ovarian follicles from the antral to the graffian stage. FSH will start a signaling cascade in the granulosa cells after sitting on its receptor (FSHR). Alteration of this receptor may change follicle maturation and therefore result in improper response to gonadotropins. We investigated the association of FSH receptor gene alteration in ovarian response of poor responder patients.

Materials and Methods: The presence of P.Ala307Thr, P.Ser680Ala, P.Ala665Thr and Mut.Val341Ala were analyzed in a case control study. 70 Iranian poor responder patients were selected as the case group. 60 Iranian fertile women were enrolled as the control group. The patients DNA were extracted from their peripheral blood and amplified by relevant primers. For determining allelic variant status all PCR products were analyzed by Sequencing.

Results: The results showed that the homozygous Ser680 and Ala307 variants seem not to be significantly associated with poor response to gonadotropins. The FSHR P. Ala665Thr genotype frequency was similar in all patients and controls. The number of oocytes retrieved was comparable between patients with different FSHR genotype.

Conclusion: Although data are accumulating with evidence suggesting that the ovarian response to gonadotropins may be mediated by different genetic alterations, the optimal biomarkers and the efficacy of the tests still remain to be evaluated.

Key words: *Female infertility, Poor ovarian response, Follicle stimulating hormone receptor (FSHR).*

P-132

A review of studies about vascular endothelial growth factor gene polymorphisms as genetic cause of infertility

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Introduction: Infertility is defined as an inability of couples to achieve pregnancy after one year of regular, non-contraception intercourse. Diverse factors such as environmental, immunological, endocrine and genetic predispositions are thought to be involved in the development of the infertility. Infertility may be due to male or female factors or both. Causes of female infertility may include ovulation disorders, uterine abnormalities and fallopian tube blockage. Recurrent miscarriage (RM) and endometriosis are the common cause of infertility in female. Genetic factors can play an important role in susceptibility to RM and endometriosis, such as polymorphisms in vascular endothelial growth factor gene (*VEGF*). Recently researchers have found that endothelial dysfunction and placental ischemia/hypoxia are important factors in miscarriage. VEGF plays a pivotal role in the formation of blood supply of the fetus through the placenta and development of endometriosis. Many recent studies have indicated that a reduced VEGF serum concentration was associated with increased risk of RM and endometriosis. Therefore, the aim of this study was to investigate the association of *VEGF* polymorphisms such as +405 G/C, -460 C/T in 5'-UTR, +936 C/T in 3'-UTR and -1154 G/A in promoter region, with RM and endometriosis.
Materials and Methods: This review is focused on the most recent laboratory and clinical findings to investigate the association of *VEGF* polymorphisms with RM and endometriosis.
Results: -1154A and +93 6T alleles are increased in RM, while only in the study of Hsieh *et al* +405 C/C and -460 T/T genotypes are highest in patients suffering from endometriosis.
Conclusion: According to many functional studies, A allele of -1154G/A, T allele of +936C/T, C/C genotype of +405 G/C and T/T genotype of -460 C/T are associated with reduced *VEGF* transcriptional levels. These could lead to inadequate angiogenesis thus, lower endometrial vascularization, impaired placentation and subsequent predispose to RM and risk of endometriosis.
Key words: *Infertility, Endometriosis, Recurrent miscarriage, Polymorphism, VEGF.*

P-133
Association study of *GSTO1* gene polymorphism (E155del) among patients with recurrent miscarriage from NorthWest of Iran

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Introduction: Recurrent miscarriage (RM) is the occurrence of three or more consecutive pregnancies that end in miscarriage of the fetus before viability. About 1-5% of couples trying to conceive are affected by recurrent miscarriage. RM is considered as a multi-factorial disease and nearly half of RM cases cannot currently be explained clinically. Several studies have indicated that genetic polymorphisms could be factors for causing susceptibility of RM with unknown etiology. GSTO1 protein has a crucial role in detoxification metabolism and it is believed that *GSTO1* gene could be involved in RM. In this study the possible association of *GSTO1* gene polymorphism [E155del (rs11509437)] with recurrent miscarriage in NorthWest of Iran was evaluated.
Materials and Methods: In this case-control study, forty-eight patients with a history of RM and forty-eight referent women, who had at least two live births without other pregnancy complications, were included. Glutathione S-transferase Omega 1 (*GSTO1*) gene polymorphism (E155del) was screened using polymerase chain reaction with confronting two-pair primers (PCR-CTPP). Subsequently, PCR products were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining.
Results: This study revealed that there was a significant association with the allelic frequency of del155 between the patient and control groups (p=0.04). However, analysis of genotype's frequencies between patient and control showed no significant association (p=0.14).
Conclusion: Although some studies have indicated that the genetic polymorphisms of detoxification genes, encoding related enzymes, could be involved in RM diseases, current study showed the weak association of *GSTO1* gene polymorphism (E155del) with susceptibility to develop RM in this population.
Key words: *NorthWest of Iran, Recurrent miscarriage, GSTO1 gene, E155del polymorphism, Cellular detoxification.*

P-134
Effects of in vitro maturation (IVM) on epigenetic changes in oocytes and early embryos

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Introduction: In vitro maturation (IVM) of oocyte is an effective technique for avoiding ovarian hyperstimulation syndrome in patients with polycystic ovaries (PCOS) during in vitro fertilization (IVF). Pervious study indicated that more than 1000 children have been born from IVM technique, especially in patient with PCOS. However, IVM remains questions even today. Epigenetic reprogramming occurs in the periods of gametogenesis and embryogenesis, which regulates the gene activity without alteration of DNA sequences. This review discuss about effects of in vitro maturation (IVM) on epigenetic changes in oocytes and early embryos.
Material and methods: This article presents result of a systematic review about effects of in vitro maturation (IVM) on epigenetic changes in oocytes and early embryos.
Results: Different studies showed that IVM down regulated the protein expression of enzymes controlling histone acetylation such as histone acetyltransferase GCN5 (GCN5) and histone deacetylase 1 (HDAC1), as well as their common target, acetyl-histone H3 (Ac-H3), in metaphase II (MII) oocytes and two-cell embryos. The significantly decreased HDAC1 mRNA levels in oocytes and early embryos from IVM strongly suggested that IVM technique down regulated the transcription of *HDAC1* gene in oocytes before and after fertilization.
Conclusion: Our studies indicated that IVM could affect the protein and gene expression level related to histone acetylation in oocytes and early embryos. Therefore, the impacts of IVM on epigenetic changes may be reasons for the lower rates of fertilization and early embryos in IVM.
Key words: *IVM, Oocyte, Epigenetic.*

P-135
Association of 14-bp insertion/deletion polymorphism of *HLA-G* gene with idiopathic recurrent miscarriage

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Introduction: HLA-G is supposed to play a pivotal role in tolerance of the semi-allogeneic graft in pregnancy by inhibiting the cytotoxic functions of T- and NK cells. A 14-bp insertion/deletion polymorphism in exon-8 has a possible role in HLA-G expression.
Materials and Methods: Genomic DNA from 200 recurrent miscarriage (RM) patients and 200 normal fertile control individuals were isolated using the routine salting out method. Exon-8 of *HLA-G* gene of the two groups were amplified using polymerase chain reaction and analyzed by electrophoresis on 10% non-denaturing polyacrylamide gel electrophoresis containing ethidium bromide and visualized under ultraviolet light. Statistical analysis used SPSS 19 software. *HLA-G* allele frequencies and genotypes in RM women and the fertile control group were compared using a Chi-square test.
Results: There was a difference in allelic frequencies of 14-bp insertion polymorphism between fertile controls and RM patients; frequency of +14/-14 bp heterozygotes was significantly increased in RM patients as compared with fertile controls.
Conclusion: A 14-bp insertion/deletion polymorphism in exon 8 plays a possible role in HLA-G expression in certain cases of recurrent miscarriage. However, additional studies are needed in this regard.
Key words: *14-bp insertion, Deletion polymorphism, HLA-G, Recurrent miscarriage.*

P-136
Mutation analysis of exone 2 of *Tnp2* gene in varicocele patients

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Introduction: Varicocele is one of the main causes of male infertility that caused by inflammation of the veins of the pampiniform plexus. Several studies have given great evidences that revealed the relationship between sperm DNA damage and varicocele. Because of the crucial role of Transition Nuclear Proteins (TNPs) and Protamines in sperm DNA condensation and integrity, the mutations in these genes can increase the risk of sperm DNA damage and infertility in varicocele condition.
Materials and Methods: DNA was extracted from total blood of 78 infertile patients with varicocele and 75 fertile control men for PCR amplification and SSCP analysis. DNA from samples with altered band pattern in the SSCP was then sequenced to search for mutations.
Results: The results of sequencing showed one variant at position IVS1-26G>C (rs8043625) in the intronic region of this gene. Comparison of the genotypes between cases and controls showed significant differences in frequencies of GG and CC (p=0.002, p=0.01), but not in GC genotype of this polymorphism (p=0.41). Also it was found that varicocele risk in men who have the CC and GC genotypes is respectively 3.07 and 1.37 fold higher than those who don't have these genotypes (OR=3.07, OR=1.37).
Conclusion: High conservation of this SNP position during evolution can represent the effects of this nucleotide in some important processes associated with the expression of this gene like mRNA splicing; but the exact mechanism is not clear.
Key words: *Varicocele, Infertility, Tnp2, SSCP.*

P-137
Assessing the polymorphism frequency rate of insulin receptor gene in PCOS

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Introduction: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder with prevalence of 5-10% globally among women of reproductive age. PCOS is being characterized by chronic anovulation, polycystic ovaries, hyperandrogenism, hirsutism, overweight, insulin resistance and infertility. It is well-known that PCOS is a complex trait like type-2 diabetes where both genetic and environmental factors play a crucial role in pathogenesis of the disease. Some type-2 diabetes susceptibility genes including those for insulin secretion and action such as insulin receptor showed considerable contribution to genetic predisposition of PCOS. Since insulin receptor gene seems to be a strong candidate gene to PCOS, we aimed to investigate the role of insulin receptor gene polymorphism (T/C) located in a transcription enhancer element of the gene to the disease susceptibility.

Materials and Methods: We carried out a cross-sectional case-control study. Using simple random sampling 90 healthy women were selected. All 90 patients fulfilled the 2003 Rotterdam criteria of PCOS. The subjects were genotyped for insulin receptor gene polymorphism (T/C) using PCR-Sequencing. Differences in genotype distributions between case and control subjects were examined via a chi-square test.

Results: In the present study 180 women between 18-40 years old age were assessed. Clinical, biochemical and metabolic characters of women were compared. Among them, obesity (Waist round more than 85 centimeters), hirsutism and irregular menarche (monthly cycles more than 35 days), LH/FSH >2, BMI>25, (mean: 28.19) in the PCOS group were significantly higher than control group ($p<0.05$). There was no significant associated between two groups for diabetes mellitus type II, hypertension (>140/90 mmHg) and hormonal level (TSH >5 and prolactin >29.2). Similarly in PCOS and control group, 95% of women had TT genotype and 2.2% had CC genotype. Frequency of C allele in PCOS and control group were 3.3% and 2.2% respectively. Frequency of TC genotype in PCOS and control group were 2.2% and 4.4% respectively. Noted polymorphism had no significant difference between PCOS and control women ($p=0.99$).

Conclusion: Although PCOS showed high prevalence among women in Bandar Abbas, Iran, insulin receptor gene polymorphism (T/C) didnot show significant influence on susceptibility to PCOS.

Key words: *Polymorphism, Insulin receptor gene, Polycystic ovarian syndrome.*

P-138

Association of SNP rs.2414096 of *CYP19* gene with polycystic ovarian syndrome in Iranian women

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Introduction: Polycystic ovarian syndrome (PCOS) is the most common endocrinologic disorder of women in their reproductive age. Major characteristics of PCOS are clinical and/ or biochemical hyperandrogenism, ovulatory dysfunction and polycystic ovaries. The etiology of PCOS is unknown, however, it has been suggested that genetic factors play major role in development of PCOS. Of those the ones implicating in metabolism of androgens are important as they are impaired in PCOS. CYP19 gene encodes aromatase which has a crucial role in androgen synthesis and variants of this gene might implicate in the pathogenesis of PCOS. Investigations have indicated the role of single nucleotide polymorphism of *CYP19* in hyperandrogenism and PCOS in some racial groups. There is no data on the variants of this gene in PCOS population in Iran.

Materials and Methods: In a case-control study 70 PCOS women and 70 normal controls were selected. Following informed consent, 5 ml blood was taken from each woman of which genomic DNA was extracted by salting out method. Then a set of PCR reactions for *CYP19* gene was carried out using specific primers for SNP rs.2414096 followed by a subsequent enzyme digest (RFLP) with *HSP92II*.

Results: Genotype frequencies of SNP rs.2414096 in PCOS women were as follows: AA (14.4%), AG (44.3%) and GG (41.4%) while in normal group, these genotypes were 24.3%, 52.9% and 22.9%, respectively. While allele frequencies in PCOS group were 49.3% for A and 50.7% for G, normal group had a different percentage of A (36.4%) and G (63.6%). The calculations for both genotype and allele frequencies showed statistical significance.

Conclusion: Variants of SNP rs.2414096 in *CYP19* could play a role in development of PCOS in Iranian women.

Key words: *Polycystic ovarian syndrome, CYP19, Single nucleotide polymorphism.*

P-139

Exposure to tertiary-butyl hydroperoxide (TBHP)- down regulates the expression of Ddx3y in mature mice testis

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Introduction: Approximately 40% of infertility is associated with male factors. Two major causative factors of male infertility are oxidative stress (OS) and genetic factors. OS damages the sperm plasma membrane, the genome integrity and alter the expression profile of genes involved in

spermatogenesis. *Ddx3y* gene is one of the important azoospermia factor (AZF) genes in Y chromosome. AZF deletion causes a severe block in spermatogenesis which affects the proliferation of spermatogonia, and consequently male infertility. The expression profile of *Ddx3y* gene was evaluated in testis tissues of Balb/c mice after OS induction.

Materials and Methods: A model of oxidative stress in adult male Balb/c mice testis by injection of the 1:10 concentration of tertiary-butyl hydroperoxide (TBHP) was created. Case group included treated mice by TBHP for 2 weeks and control group treated only by injection of dH_2O. Induced ROS levels in testes tissue samples of all mice were measured by flow-cytometry. Consequently the expression of *Ddx3y* gene was quantitatively measured in samples of both groups by real-time PCR.

Results: According to flow-cytometry results, an increase of oxidative stress in TBHP treated mice in comparison to control group was observed. The gene expression of Ddx3y in testis was significantly down regulated in OS-exposed and ROS induced mice.

Conclusion: Our results indicated that *Ddx3y* may be a major target gene of OS and the down regulated expression of Ddx3y can be closely related to male reproductive toxicity induced by TBHP.

Key words: *Male infertility, Oxidative stress, TBHP, Ddx3y.*

P-140

Association of +331G/A polymorphism and differential expression of PR-B isoform in endometriosis

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Introduction: It is well accepted that endometriosis is a progesterone resistance disease. The effects of progesterone are mediated by its two progesterone receptor (PR) isoforms, namely PR-A and PR-B. These isoforms are functionally different. The most widely studied polymorphism in the promoter region of *PGR* is the G to A substitution at position +331 (rs10895068). Recently, many studies have investigated the role of +331G/A polymorphism in the etiology of various types of cancers.

Materials and Methods: Blood samples were recruited from 98 women undergoing laparoscopy for endometriosis and 102 healthy fertile women at Royan Institute, Tehran, Iran in 2013-2014. After DNA extraction, allele and genotype frequencies were determined by PCR-RFLP. Then, RNA was extracted from selected eutopic tissue samples of endometriosis patients. Analysis of PR-B mRNA expression was performed using Real-time PCR.

Results: Our data showed the frequency distribution of GG, G/A and AA genotypes in +331G/A polymorphism was 98.04%, 1.96% and 0.0% in patients and 97.96%, 2.04% and 0.0% in control groups respectively (p=0.968). Although our data didn't show any significant association with +331G/A in our groups, however, we were able to demonstrate higher expression level of PR-B in patients with G/A compared to patients with GG genotypes.

Conclusion: Many studies have been shown that the expression level of PR-B reduced severely during endometriosis, which can affect the function of progesterone. Our findings support this observation, patients with G/A genotypes have high expression level of PR-B compared to patients with GG genotypes. Therefore, +331G/A have been found to lead increased transcriptional activity of PR-B in patients by favoring G/A or AA genotypes that may be able to influence the function of progesterone and reduce the susceptibility and symptoms of endometriosis.

Key words: *Endometriosis, PGR, Polymorphism.*

P-141

Association between *GSTT1* null mutation and endometriosis in an Iranian population

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Introduction: Endometriosis is a common gynecological disease that involves growth of endometrial tissue outside its normal location. It seems that genetic factors have an important role in the pathophysiology of endometriosis. In addition, there are reports suggesting a role for environmental pollutants in developing endometriosis. GSTTl is one of the phase II enzymes involved in detoxification of some environmental carcinogens.

Materials and Methods: Genomic DNA from 95 patients with endometriosis and 141 healthy controls was collected. *GSTT1* null genotyping was performed using polymerase chain reaction (PCR) method.

Results: Frequency of *GSTT1* null deletion in both patients and controls was 22%, which shows no significant difference between two groups (p=0.983).

Conclusion: These results suggest that GSTT1 null mutation is not associated with endometriosis in our population.

Key words: *Endometriosis, Glutathione s-transfers, GSTT1.*

P-142

The low frequency of breast and ovary cancer protective allele (D302H) of *CASP8* gene among Iranian patients

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Introduction: Suppression of apoptosis is one of the major mechanisms underlying the origin and progression of cancer. The CASP8 plays a key role in the initiation of apoptosis. Previous case-control studies have indicated that the *D302H* genotype of *CASP8* is associated with a reduced risk of breast and ovarian cancer. However, the frequency of this polymorphism in the Iranian people has not been reported yet. Therefore, this polymorphism was genotyped among patients with breast cancer and healthy women.
Materials and Methods: PCR-RFLP technique was used to genotyping of *CASP8 D302H* polymorphism among 100 female patients with breast cancer and 100 healthy women in this case-control study. Fisher's exact test and SPSS software were used to analyze the data.
Results: The mean age of patients and healthy groups was 51.37±1.27 and 52.57±1.21 years respectively. The frequencies of the GG and GC genotypes in case group were 91% and 9%, respectively, and among the control group, the frequencies were 97% and 3%, respectively (p=0.2). CC genotype was not found in any of the groups. Furthermore, the C allele frequency was 4.5% among the cases and 1.5% among the control (p=0.14). There was no significant difference between cases and controls. In total, the frequency of the C allele was observed in about 3% of people in our study (breast cancer patients and healthy women).
Conclusion: Previous case-control studies have indicated that the *D302H* genotype of *CASP8* was associated with a reduced risk of breast and ovary cancer. In the present study the frequency of allele C was detected 3%. Indeed, the frequency of C allele in Iranian study population is less than one-fourth the frequency of this allele in Caucasian populations (13.29%). According to the low frequency of this allele in Iranian population and the protective effect of allele C, one of the conclusions that can be raised is that the Iranian population is more prone to breast and ovarian cancer than Caucasian populations in relation to this polymorphism. Therefore further studies would be needed with appropriate sample size to investigate this matter.
Key words: *CASP8 D302H, Polymorphism, Apoptosis, Breast cancer.*

P-143
"Cell-free fetal DNA" a novel promising biomarker for prenatal diagnosis and complicated pregnancies

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Introduction: Cell free fetal DNA (cff DNA) is a novel promising molecular biomarker that has been applied in various aspects of obstetrical research, notably in prenatal diagnosis and complicated pregnancies.
Materials and Methods: Data base was browsed using key words "cell-free fetal DNA" and "noninvasive prenatal diagnosis" for obtaining related reports. Articles were screened for relatedness and the recent progresses in the field.
Results: Cell-free fetal nucleic acids can be detected in the maternal circulation during pregnancy, potentially offering an excellent method for early non-invasive prenatal diagnosis (NIPD) of the genetic status of a fetus. Using molecular techniques, fetal DNA and RNA can be detected from 5 weeks gestation and are rapidly cleared from the circulation following birth. Cell-free fetal DNA comprises only 3-6% of the total circulating cell-free DNA, therefore diagnoses are primarily limited to those caused by paternally inherited sequences as well as conditions that can be inferred by the unique gene expression patterns in the fetus and placenta. Broadly, the potential applications of this technology fall into two categories: first, high genetic risk families with inheritable monogenic diseases, including sex determination in cases at risk of X-linked diseases and detection of specific paternally inherited single gene disorders; and second, routine antenatal care offered to all pregnant women, including prenatal screening/diagnosis for aneuploidy, particularly Down syndrome (DS), and diagnosis of Rhesus factor status in RhD negative women. Already sex determination and Rhesus factor diagnosis are nearing translation into clinical practice for high-risk individuals.
Conclusion: In conclusion, the discovery of Cell free fetal DNA (cff DNA) has revolutionized the field of non-invasive prenatal diagnosis (NIPD) and has opened a new avenue in the field of obstetrical research and may in future form part of national antenatal screening programmes for DS and other common genetic disorders.
Key words: *Cell free fetal DNA, Noninvasive prenatal testing (NIPT), Prenatal diagnosis, Pregnancy complications.*

P-144
Identification of a time window for spontaneous establishment of pluripotency in mouse spermatogonial stem cells in vitro

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Introduction: Although testis-derived embryonic stem cell-like (ES-like) cells have been obtained in several studies, the time window for the shift to pluripotency is not clear yet.
Materials and Methods: Here we describe, that only during a special time window (41 until 125 days) after initiation of germ line stem cell (GSCs) cultures from neonate and adult promoter-reporter Oct4-GFP transgenic mouse the spontaneous appearance of germline-derived pluripotent stem (gPS) cells from both neonate and adult GSCs occurred.
Results: The isolated and long-term cultured (more than one year) GSCs which were isolated by a morphology based selection procedure expressed germ cells markers and exhibited a similar morphology with a high nucleus/cytoplasm ratio in comparison to undifferentiated SSCs (spermatogonial stem cells) *in vivo*. The generated gPS cells expressed pluripotency marker, in-vitro differentiated into all three germ lineages, formed complex teratoma after transplantation in SCID mice and produced chimeric mice.
Conclusion: Although the exact mechanism of the development of gPS cells from GSCs is still unclear, this new information could provide an ideal strategy for scheduling natural conversion mechanisms of ES-like cells from mouse testis.
Key words: *Germ cells, Spermatogonial stem cells, Pluripotency.*

P-145
Association between MspI polymorphism of *CYP1A1* gene and endometriosis in an Iranian population

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Introduction: Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity. It is associated with dyspareunia, dysmenorrhea, pelvic pain and infertility and about 3-10% of women suffer from it in reproductive age. It seems that genetic and environmental factors play a role in the development of endometriosis. The relationship between genetic polymorphisms and endometriosis has been investigated in several genetic studies. *CYP1A1* gene is a member of the cytochrome P450 gene family. It is involved in estrogen metabolism and in detoxification.
Materials and Methods: In a case-control study, DNA extraction was performed from the blood specimens of 93 cases of endometriosis and 139 healthy controls. The polymorphism was determined by PCR-based restriction fragment length polymorphism (RFLP) method.
Results: There was no significant difference in frequency of TT, TC, and CC genotypes of MspI polymorphism of *CYP1A1* gene between case and control groups (p=0.961).
Conclusion: Results of this study revealed no association between the MspI polymorphism of the *CYP1A1* gene and susceptibility to endometriosis in the studied population.
Key words: *Endometriosis, MspI polymorphism, CYP1A1 gene.*

P-146
Preimplantation genetic screening: Which stage? Which technique?

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Introduction: Preimplantation genetic screening (PGS) techniques have a wide range of resolution, complexity and costs. Additionally each of biopsy stages has advantages and disadvantages.
Materials and Methods: Recent publications certainly systematic reviews and randomized clinical trials were considered in details.
Results: Polar body biopsy (PB) is the least invasive however provides the least capture of abnormalities. PB focuses on female meiotic errors, fails to identify approximately 40% of aneuploidies from postzygotic and paternal origins. PB is suitable in countries with legal restrictions for embryo biopsy. Cleavage stage biopsy (CB) of few cells (1 or 2) does not affect differentiation potential, however removal of 2 cells can result in an impaired implantation potential while mosaicism is not detectable in one cell biopsy. In trophectoderm biopsy (TB) several cells are available for analyses. In day 5 TB when the genetic results cannot be obtained within 24 hours, cryopreservation should be considered. The least and the most aneuploidy rates has been reported for TBs and CBs, respectively (1 versus more than 3). TB increases absolute implantation rate about 20% in comparison with CB. Blastocoel fluid aspiration is a new source of DNA for PGS with few publications. FISH-based PGS is low resolution due to limitation in number of both chromosomes and probes per chromosome which could be tested. Therefore it has not been effective in improvement of clinical outcomes while PGS methods with ability for detection of all 24 chromosomes (PGS-24) result in increased implantation and pregnancy rates and diminished effect of advanced maternal age. Concerning PGS-24 methods, digital PCR is mostly applied for PBs; real time qPCR is only applicable to TBs and limited by number of samples, two on each 384-well plate. Array CGH is robust and scalable, turnaround time as short as 12 hours, decreased cost per sample as increased case numbers.

SNP arrays and NGS-based methods for copy number analysis are likely to be the most accurate and informative but complex and high-cost equipment. Low pass NGS-based PGS is moving to be cost effective in addition to performance same as array CGH.

Conclusion: Clearly, the largest reported increases in implantation and live birth rates to date have been with TB. TB is a good choice for good-prognosis patients particularly for those wishing to have elective single-embryo transfer. However, the cleavage-stage embryos of some poor-prognosis patients may implant and develop in utero but not develop to the blastocyst stage in vitro. At the moment, array CGH seems to be the most applicable method for PGS however future would be with higher resolution methods following decrease of related costs, hence ethical concerns on designer babies will need more serious considerations.

Key words: *PGS, Biopsy, Stage, Technique.*

P-147

Expression of CYP19A1 in patients with endometriosis and normal endometrium during the menstrual cycle

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Introduction: Endometriosis is an estrogen-dependent inflammatory disease defined by the growth of endometrial tissues outside of the uterus. Epidemiological and clinical studies show that estrogen is essential for the growth of endometriosis. Molecular studies have revealed the presence of aromatase P450, the key enzyme in the biosynthesis of ovarian estradiol, inside the endometriotic tissues, indicating local synthesis of estradiol. So, it is proposed that the enzyme aromatase P450, which is coded by *CYP19A1* gene, will be expressed aberrantly in endometriosis.

Materials and Methods: To evaluate the expression profile of *CYP19A1* gene in women with endometriosis during menstrual cycle, ectopic endometriotic lesions and eutopic endometrium samples were collected using laparoscopy from 20 women with endometriosis and endometrial biopsies were obtained from 10 healthy fertile women as a control group. For this respect, ethical approval and informed patient consent was gained for the use of tissue samples. Quantitative expression analysis was performed using real-time PCR technique.

Results: Data showed that mRNA expression of CYP19A1 was significantly higher in ectopic endometrium of patients with endometriosis, in both proliferative and secretory phase, in comparison with eutopic and control group. CYP19A1 expression in eutopic endometrium was significantly higher in comparison to control group in proliferative phase.

Conclusion: Higher expression of CYP19A1 mRNA in the endometriotic tissues, may contribute to the etiology and progression of endometriosis and it is involved in disease pathogenesis.

Key words: *Endometriosis, CYP19A1, Menstrual cycle.*

P-148

A headache in prenatal diagnosis: A case of vanished twin or hermaphroditism?

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Introduction: The finding of a mixture of both 46,XX and 46,XY cells in amniotic fluid culture has been frequently described. In the great majority of cases, the finding is followed by the birth of a normal male infant, leading to a consensus that the finding is the result of contamination with maternal cells in a normal male fetus. There are, however, several other possible explanations including the presence of cells from an undiagnosed twin pregnancy, cross contamination in the laboratory, the presence of cells from a "vanished" male twin, and true fetal chimerism. An accurate obstetric history and thorough sonography can be of great value in correct assessment of such situations.

Materials and Methods: A 29 Years-old woman was referred for genetic counseling. Her gestational age was 16 weeks and maternal biochemical serum screening test indicated high risk for Down Syndrome. She underwent amniocentesis for chromosome study using standard high resolution GTG banding technique.

Results: Karyotype result was 46,XY and sonography revealed a female fetus with normal internal genital organs. A normal baby girl was borned with 46,XX karyotype. Upon reviewing of amnio GTG slides both 46,XY and 46,XX cell lines were detected. The patient admitted that she had experienced a miscarriage and heavy bleeding early in pregnancy. The sonography at the time of amniocentesis had shown a shrinking cyst next to the fetus. These findings strengthened the vanished or resorbed twin as the reason for such a finding.

Conclusion: The fetus was most likely a non-identical twin. Most of grown cells were from the vanished male fetus which led to misdiagnosis. Thorough genetic

counseling with the view of obstetric history is of great value in such situations.

Key words: *Amniocentesis, Resorbed twin, Hermaphroditism, Genetic counseling.*

P-149
+49A/G *CTLA-4* gene polymorphism in NorthWest Iranian population with recurrent pregnancy loss

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Introduction: Recurrent pregnancy loss is defined as three or more pregnancy loss before 20th gestational weeks. There are several factors which involved in pregnancy loss including anatomical, hormonal, immunological and infectious factors. Immunological factors are important due to the interaction between mother and fetus. In spite of allogeneic proteins encoded by paternal genes, it is clear that a series of regulatory mechanisms should exist in maternal immune system not to reject fetus during pregnancy. T regulatory cells which are one of the important component of humeral immune response plays role in the fetu-maternal interface. One of the regulatory ways for these cells is mediated by antigen independent co-stimulatory signals. Interaction of B7/ CTLA-4 is one of these signals. Cytotoxic T Lymphocyte Antigen 4(CTLA-4) is a glycoprotein transmembrane molecule which down regulate the activation and proliferation of T cells in a competitive interaction with CD28 to bind to B7.
Materials and Methods: We have studies 120 cases; patient group consist of 60 women with the experience of two or more pregnancy loss and control group consist of 60 women with at least two live births without any previous history of pregnancy loss and autoimmune diseases. Genomic DNA was extracted from whole blood using standard protocols. The *CTLA-4* +49 A/G were detected using polymerase chain reaction-restriction fragment length polymorphisms assay.
Results: In this study *CTLA-4* +49 A/G polymorphisms showed no significant differences between patients and controls. Polymorphic allele G showed the frequency of 39.16% among patients whereas its frequency for controls was 35.83%. The age of patient varied from 19 years to 39 years.
Conclusion: The results of the present study suggest that the CTLA-4 do not have association with recurrent miscarriage in NorthWest Iranian population.

Key words: *Recurrent pregnancy loss, CTLA-4, Immunological factors.*

P-150
Investigation of mitochondrial Gln tRNA molecular alterations in idiopathic repeated pregnancy loss

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Introduction: Recurrent pregnancy loss (RPL) is a critical medical problem in about 0.5-2% of women. The molecular genetics background for spontaneous abortion is being increasingly understood, and some polymorphisms associated with it have been reported
Materials and Methods: The nucleotide variations of threonine and proline were investigated in 96 women with idiopathic repeated pregnancy loss. The related mitochondrial area was amplified using a polymerase chain reaction (PCR). The PCR products were demonstrated by 2% agarose gel electrophoresis, and all the positive samples were purified and verified by an automated DNA sequencing method.
Results: The sequence analysis revealed 2 mutations in tRNA Gln . These mutations were 4343 in 1 cases and 4336 in 1 cases .
Conclusion: These tRNAs mutations can alter their steady state level and affect the structure of tRNAs. It results in protein synthesis defects and, in turn, mitochondrial dysfunction. The mutations of these genes may help in the assessment of RPL. Further study of an expanded series of these tRNA mutants is recommended to describe their etiologic role in idiopathic RPL.

Key words: *tRNA, Mitochondrial mutation, Repeated pregnancy lo.*

P-151
A novel mutation in morquio syndrome

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Introduction: The Mucopolysaccharidoses (MPS) are a group of inherited metabolic disorders caused by a deficiency or malfunctioning of lysosomal enzymes which are needed to break down complex carbohydrates known as mucopolysaccharides or glycosaminoglycans (GAGs). Accumulation of GAGs causes a cascade of events leading to the progressive damage of cells, tissue and organs. Morquio disease or Mucopolysaccharidosis Type IV (MPS IV) belongs to this group and has two sub-types, A and B. Type A is also known as Morquio A, GALNS deficiency, N-acetylgalactosamine-6-sulfate sulfatase deficiency or more simply MPS IVA. This lysosomal enzyme involved in the catabolism of keratan and chondroitin sulfate. Patients who inherit two mutated *GALNS* gene alleles have a decreased ability to degrade the glycosaminoglycans (GAGs) keratan sulfate and chondroitin 6-sulfate, thereby causing GAG accumulation within lysosomes and consequently pleiotropic disease. The objective was to report the

results of clinical characteristics, enzyme activity determination and mutation analysis of *GALNS* gene in an Iranian patient with mucopolysaccharidosis (MPS) type IVA (Morquio A disease).

Materials and Methods: The 10 years old Iranian boy with MPSIV was firstly diagnosed by urin GALNS determination who was charactraised by multiple skeletal abnormalities and dwarfism, kyphosis, hypermobility joints, difficultly in walking to 8 years old and now he can't walk. But his intelligence was normal. We investigated for all coding exons and adjacent intron regions of *GALNS* gene by PCR sequencing method.

Results: We performed urin test (MS/MS) from patient. In this test total GAGS was high and MPS was likely. So lysosomal enzymes in dried blood were checked and MPS IVA was diagnosed. Two heterozygous missens mutation as c.135 G>T (S>I) and c.510 C>A (P>H), and a homozygous mutation as c.181 A>G (y>C) in *GALNS* gene were detected in this patient. The mutation c.181 (y>C) is a novel variant which is not reported yet.With the method of gene analysis of new variant, the mutation c.181 (y>C) was considered to be a pathogenic mutation.

Conclusion: The MPS IVA patient showed severe multiple skeletal deformities, normal intelligence, muscle weakness, short stature, who carries homozygous mutations c.181 A>G (y>C). The bioinformatics analysis in POLYPHEN predicted this mutation as being probably pathogenic.

Key words: *Mucopolysaccharidoses (MPS), Morquio disease, GALNS gene.*

P-152

A newborn with ambiguous genitalia and a complex X;Y rearrangement

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Introduction: In most mammals, sex is determined at the beginning of gestation by the constitution of the sex chromosomes, XY in males and XX in females.

Materials and Methods: Array Comparative Genomic Hybridization (Array-CGH) analysis was performed by using oligonucleotide aCGH platforms (180K SurePrint G3 Human Kit, Agilent Technologies, Santa Clara, CA), as reported elsewhere. Changes in DNA copy number at a specific locus were observed as the deviation of the log2 ratio value from 0 of at least three consecutive probes, by using Genomic Workbench v. 5.0.14 software (Agilent, ADM-2 algorithm with a threshold of 5). Oligomer positions refer to the Human Genome GRCh37 (hg19) assembly.

Results: Array-CGH revealed an unbalanced rearrangement resulting in the deletion of the distal Xp and the duplication of the proximal Xp contiguous region with presence of the Y chromosome from Ypter to Yq11. Fluorescent in situ hybridization (FISH) showed that this portion of the Y was translocated to the tip of the abnormal X and that the duplicated portion of chromosome X was inverted. Altogether, the abnormal chromosome was a dicentric one with the centromere of the Y chromosome apparently inactivated.

Conclusion: The presence within the translocated Y chromosome of the SRY gene explains the devolopment of testes although it is not clear the reason for the genitalia ambiguity.

Key words: *Ambiguous genitalia, 46, XX testicular DSD, Inverted duplication and Xp terminal Deletion (Invdup del), Rearrangement, Array Comparative Genomic Hybridization, FISH.*

P-153

Effect of genetic variation of beta defensin 126 on ICSI and IVF outcome in unexplained infertile men

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Introduction: Despite improved methods for evaluation of sperm quality, infertility remains unexplained in about 20% of affected couples. During sperm maturation, a Cysteine-rich secretory glycoprotein α-defensin 126 secreted by the epididymal epithelium adsorbed to the entire sperm surface. It remains on the sperm until sperm become capacitated in the female reproductive tract. Its removal from over the head of sperm is required for sperm zona recognition. A cytosine dinucleotide deletion in the open reading frame of second exon of *DEFB126* gene generates an abnormal mRNA. Men homozygous for this mutation have reduced chances of successful fertilization.

Materials and Methods: Genomic DNA from the peripheral blood of 92 male partners of unexplained couples who underwent ICSI (n=74) and IVF (n=18), were extracted. PCR was performed and molecular genotyping for the *DEFB126* variant was done by single strand conformational polymorphism (SSCP), tetra PCR and DNA sequencing. ELISA and immunocytochemistry by indirect immunofluorescence antibody performed for the assessment of this protein expression on sperm cells.

Results: In our study this allele frequency in Iranian men was 0.54. Statistical analysis shows, no significant differences were found between homozygote mutation and wild type carriers in fertilization rates, implantation rates and clinical pregnancy of IVF and ICSI. Our results by ELISA and immunocytochemistry showed that the protein expression was less in men with del/del genotype in comparing to other genotypes ($p<0.005$).

Conclusion: Although previous studies found that *DEFB126* variation would affect sperm function and male fertility rate, in the present study, no significant

differences were found between homozygote mutation and wild type carriers in fertilization rates, implantation rates and clinical pregnancy. Further confirmation in a larger scale study is needed.

Key words: *Unexplained male infertility, Deletion, DEFB126, IVF, ICSI.*

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Association of *TNFR1* 36 A/G polymorphism with azoospermia in Iranian infertile males

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Introduction: Infertility is the failure to achieve a pregnancy after one year of regular sexual intercourse without contraception. Half of the cases of infertility are due to male factors and nearly 60-75% of reasons of male infertility are unknown, due to unknown mechanisms of molecular defect. Tumor Necrosis Factor α (TNF-α) is a multi-functional cytokine that regulates cellular processes related to spermatogenesis. Tumor Necrosis Factor Receptor 1 (TNFR1) mediate TNF-α activity and changes in its structure can affect TNF-α activity. Studies show that variation in the *TNFR1* gene may be associated with male infertility.

Materials and Methods: This case-control study includes 108 azoospermic men and 119 healthy controls. We investigated the association of *TNFR1* 36 A/G in the population with idiopathic azoospermia who referred to Yazd Institute of Reproduction Sciences. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism **(**PCR- RFLP) method was used to genotype the single nucleotide polymorphism in the both groups. PCR fragments digested by enzymes Mspa1I, and were separated by gel electrophoresis and then the frequency of A→G substitution in azoospermic males and fertile men were counted.

Results: According to our study, G/G genotype in the control group has higher frequency among men with azoospermia [p=0.01; OR=2.29 (1.248-4.229)]. Our findings also show that the allele frequency of G allele in azoospermic men is significantly different in comparison with control group [p<0.001; OR=2.302 (1.580-3.55)].

Conclusion: It seems that the G/G genotype and G allele are significantly associated with increased risk of idiopathic azoospermia.

Key words: *Polymorphism, Male infertility, Sperm, Cytokines, Tumor necrosis factor alpha.*

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Epigenetic evaluation of histone methylation on *HOXA1-5* genes in cumulus cells of polycystic ovary syndrome patients

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Introduction: Polycystic ovary syndrome (PCOS) is the major cause of an ovulatory infertility with uncertain etiology. Epigenetics and environment play critical roles in PCOS. Of important genes correlated with human reproductive system disorders are HomeoboxA (*HOXA*) cluster genes. Regarding the epigenetic role of histone modifications in regulation of gene expression, lysine (K) methylation of histone3 (H3K9me), as a repressive epigenetic mark, on the promoter regions of *HOXA1-5* genes were evaluated in this study. For this respect, cumulus cells (CCs) which have critical roles during folliculogenesis, oocyte maturation, ovulation and fertilization, were aimed to monitor expression profile and epigenetic alterations of *HOXA1-5* genes.

Materials and Methods: CCs were collected from 20 PCOS patients and 20 fertile women (18-36 year) with male infertility problems, referred to Royan Institute to have IVF-ICSI under GnRH antagonist protocol. Informed consents were obtained from the participants. After evaluation of genes expression by qRT-PCR, chromatin immune precipitation (ChIP) coupled with real-time PCR was performed to evaluate incorporation of H3K9me into regulatory regions of *HOXA* genes.

Results: Expression data, revealed significant decrease in mRNA level of HOXA1 (p<0.05) and significant increase in HOXA2, HOXA3, HOXA5 (p<0.05) in cumulus cells of PCOS patients vs. control group. There was no significant change in expression level of HOXA4 (p<0.05) among two studied groups. Obtained data from ChIP Real-time PCR verified well the results of gene expression; in the way that histone methylation level in regulatory region of *HOXA1* does not show any alteration but there were significant decrease of H3K9me in promoters of *HOXA2, HOXA3, HOXA4* and *HOXA5* (p<0.05), in PCOS patients vs. control group.

Conclusion: These findings demonstrate the significant association of aberrant histone methylation with impaired oocyte maturation and confirm the functional role of epigenetics in pathogenesis of PCOS.

Key words: *HOXA1-5, Histone methylation, Epigenetic, PCOS.*

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