Key Lectures

K-1
New treatment in PCOS

Salehpour S, Nazari L.
Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: saghar.salehpour2014@gmail.com, somaye.rastkar@yahoo.com

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

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

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

K-2
Endocrine disrupting chemicals and male infertility

Dohle GR.
Erasmus MC Rotterdam, The Netherlands.
Email: g.r.dohle@erasmusmc.nl

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

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

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

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

Saito Sh, Shima T, Nakashima A, Yoshino O.
Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan.
Email: s30saito@med.u-toyama.ac.jp

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

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

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

Soygur B[1, 2], Moore H.D.[1].
1. *Centre for Stem Cell Biology, University of Sheffield, S10 2TN, UK.*
2. *Akdeniz University, School of Medicine, Department of Histology and Embryology, Antalya, Turkey.*

Email: h.d.moore@sheffield.ac.uk

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

K-5
Advanced cellular technologies in reproductive biology

Hamidieh AA.
Council for Development of Stem Cell Sciences and Technologies, Vice Presidency of Science and Technology, Tehran, Iran.
Email: aahamidieh@sina.tums.ac.ir

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

K-6
The transition from genetic to genomic medicine

Romeo G.
European School of Genetic Medicine and Alma Mater Studiorum, University of Bologna, Italy.
Email: egf.giovanni.romeo@gmail.com

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

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

K-7
When ART is indicated in endometriosis

Moini A[1,2].

1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Gynecology and Obstetrics, Roointan Arash Women's Health Research and Educational Hospital, Tehran University of Medical Sciences, Tehran, Iran.*

Email: a_moini@royaninstitute.org

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

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

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

Fazeli A.
1. *Academic Unit of Reproductive and Developmental Medicine, The Medical School, University of Sheffield, United Kingdom.*
2. *Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Estonia.*

Email: a.fazeli@sheffield.ac.uk

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

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

Rahimi M.
Department of Obstetrics and Gynaecology, Grey Base Hospital, Greymouth, New Zealand.
Email: Mahmoud.rahimi@health.nsw.gov.au

Abstract not received.

K-10
ART in PCOS patient

Aleyasin A.
Department of Infertility, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: ivfshariati69@gmail.com

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

K-11
Laparoscopic myomectomy, for unusual myoma locations

Asefjah H.
Parsian Hospital, Tehran, Iran.
Email: armina.asf@gmail.com

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

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

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

K-12
Treatment option in PCO resistant to clomiphene citrate

Aghahoseini M.
Department of Infertility, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: ivfshariati69@gmail.com

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

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

Zuffardi O[1], Kurtas EN[1], Dehghani MR[2, 3].
1. *Department of Mulecular Medicine, University of Pavia, Pavia, Italy.*
2. *Medical Genetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: orsetta.zuffardi@unipv.it

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

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

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

Al-Hasani S.
IVF Consulting, Vorwiesenweg 7, 23560 Luebeck, Germany.
Email: al-hasani@ivf-consulting.com; sf_alhasani@hotmail.com

Abstract not received.

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

Nottola SA[1], De Santis L[2], Familiari G[1], Macchiarelli G[3].
1. *Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University, Rome, Italy.*
2. *San Raffaele Scientific Institute, Ob/Gyn IVF UNIT, Milan, Italy*
3. *Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy.*

Email: stefania.nottola@uniroma1.it

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

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

Humaidan P.
The Fertility Clinic, Skive Regional Hospital, Denmark.
Faculty of Health Aarhus University, Denmark.
Email: peter.humaidan@midt.rm.dk

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

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

K-17

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

Aflatoonian A[1, 2], Karimzadeh Meybodi MA[1, 2], Yousefnejad F[2], Taheripanah R[2, 3], Hosseini E[2, 4], Nikmard F[2, 5], Amjadi FS[2, 5], Hamidi D[2], Kariminejad R[6], Aflatoonian B[2, 7, 8, 9], Aflatoonian R[2, 10].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Assisted Conception Unit, Laleh Hospital, Tehran, Iran.*
3. *Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
4. *Department of Obstetrics and Gynecology, Shahrekord University of Medical Sciences, Shahrekord, Iran.*
5. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
6. *Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran.*
7. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
8. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
9. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
10. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: abbas_aflatoonian@yahoo.ir

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

K-18

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

Palmerini MG[1], Nottola SA[2], Anindito TW[3, 4], Bianchi S[1], Sato E[4], Macchiarelli G[1].

1. *Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy.*
2. *Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, La Sapienza University, Rome, Italy.*
3. *Faculty of Biology, Gadjah Mada University, Indonesia.*
4. *Laboratory of Animal Reproduction, Graduate School of Agricultural Sciences, Tohoku University, Sendai, Japan.*

Email: mariagrazia.palmerini@univaq.it

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

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

Karimi-Zarchi M, Forat-Yazdi M, Nakhaie-Moghadam M, Miratashi-Yazdi A, Teimoori S, Dehghani-Tafti A.

Shahid Sadoughi University of Medical Science, Yazd, Iran.
Email: Drkarimi2001@yahoo.com

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

K-20
Endometriosis in adolescence

Karimzadeh Meybodi MA.
Department of Obstetrics and Gynecology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: makarimzadeh@yahoo.com

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

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

K-21
Influence of ovarian endometrioma on fertility

Mehdizadeh Kashi A, Chaichian S.
1. *Endometriosis and Gynecologic Disorder Research Center, Iran University of Medical Sciences, Tehran, Iran.*
2. *Minimally Invasive Techniques Research Center in Women, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.*

Email: amehdizadekashi@yahoo.com

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

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

Alborzi S.
Gynecologic Endoscopy Division, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: alborzisa@gmail.com

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

K-23
Endometrial receptivity in endometriosis

Arefi S.
1. *Erfan Hospital (Givar) Infertility Center, Tehran, Iran.*
2. *Bahman Hospital Infertility Center, Tehran, Iran.*

Email: soheilaarefi@yahoo.com

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

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

K-24
Adenomyosis and infertility

Parsanezhad ME.
Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: parsame@rogers.com; parsanezhad@msn.com

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

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

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

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

Taheripanah R.
Department of Obstetrics and Gynecology, Shahid Beheshti University of Medial Sciences, Tehran, Iran.
Email: taheripanahf@gmail.com

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

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

Aflatoonian B[1, 2, 3], Sadeghian-Nodoushan F[1], Fesahat F[4], Soleimani M[4, 5], Khalili MA[4], Heidarian Meimandi H[6], Farashahi Yazd E[1], Nikukar H[1, 3], Aflatoonian A[4].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5. *Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
6. *Recurrent Abortion Reseasrch Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: b.aflatoonian@ssu.ac.ir

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

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

Arjmand B[1, 2], Goodarzi P[2], Mohamadi-Jahani F[2].

1. *Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Research Institute, Tehran University of Medical Sciences, Tehran, Iran.*
2. *Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
3. *Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.*

Email: arjmand_itb@yahoo.com

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

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

Aghayan HR.

Regenerative Medicine Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran.

Email: hr.aghayan@ gmail.com; shraghayan@sina.tums.ac.ir

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

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

Moore H.D.

Centre for Stem Cell Biology, Department of Biomedical Science, University Sheffield, UK.
Email: h.d.moore@sheffield.ac.uk

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

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

Sisakhtnezhad S[1], Matin MM[2], Bahrami AR[2].
1. *Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.*
2. *Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.*

Email: ar-bahrami@um.ac.ir

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

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

Nematollahi-mahani SN.
Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
Email: nematollahimahani@yahoo.com

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

K-32
Regenerative medicine in the reproductive system

Nikukar H[1, 2].
1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: habibnik@ssu.ac.ir

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

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

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

Sedlmayr P[1], Zardoya-Laguardia P[1], Blaschitz A[1], Wadsack C[2], Frank S[3].
1. *Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria.*
2. *Department of Obstetrics and Gynaecology, Medical University of Graz, Austria.*
3. *Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria.*

Email: peter. sedlmayr@medunigraz.at

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

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

Moazzeni SM.
Immunology Department, Tarbiat Modarres University, Tehran, Iran.
Email: smoazzeni@gmail.com

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

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

K-35
New aspects of thin endometrium in ART

Eftekhar M.
Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: eftekharmaryam@yahoo.com

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

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

Sedighi Gilani MA.
Department Of Urology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Email: masadighi@gmail.com

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

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

Macchiarelli G[1], Nottola SA[2].
1. *Department of Life, Health and Environmental Sciences, University of L´Aquila, L'Aquila, Italy.*
2. *Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University, Rome, Italy.*

Email: gmacchiarelli@univaq.it

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

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

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

Zarnani AH.
Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
Email: zarnani@avicenna.ac.ir, zarnania@gmail.com

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

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

Rezaei A.
Immunology Department, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: rezaei@mui.ac.ir; rezaei1335@yahoo.com

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

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

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

Ghasemi N.
Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: nghasemi479@gmail.com

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

Award Winners *(Alphabetic order)*

A-1

Studying Tribbles-2 role in embryo implantation and modulation of TLR5 signaling pathway in the female reproductive tract

Basatvat Sh[1], Carter D[2], Maslehat Lay N[1], Elliott S[1], Kiss-Toth E[3], Fazeli A[2, 4].

1. *Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.*
2. *St James' Biomedical Services, University of Leeds, Clinical Sciences Building, St James' Hospital, Beckett Street, Leeds LS9 &7TF, UK.*
3. *Department of Infections, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield S10 2RX, UK.*
4. *Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Tartu, Estonia.*

Email: s.basatvat@sheffield.ac.uk

Introduction: Successful embryo implantation is a compulsory yet cryptic episode in reproduction. The maternal innate immune system, specifically the Toll-Like Receptors (TLRs) as the main family of pathogen recognition receptors, is involved in maintaining the immunity in the female reproductive tract. Activation of TLRs with their specific ligands, during the implantation process has a negative effect on the implantation outcome. Tribbles proteins, a family of psuedokinase proteins, modulate various signaling pathways within the cell. Tribbles-2 (Trib2) protein is modulating TLR5 signal transduction pathway. We have shown that Trib2 knockout female mice were infertile. Hence, we hypothesized that Trib2 protein is involved in regulation of female fertility. Initial investigations demonstrated that embryo implantation failure might be the main cause of infertility in the female Trib2 knockout mice. Thus, the aim of this study is to understand the role of Trib2 in embryo implantation and regulation of TLR5 signaling pathway and as a result how this protein is involved in the female fertility.

Materials and Methods: To investigate Trib2 protein involvement in embryo implantation, wild-type mouse embryos were transferred into the oviducts of Trib2 null, Trib2 heterozygotes and wild-types. Furthermore, the desired combination of the functional TLR5 signaling pathway and the functional Trib2 protein in different human endometrial cell-lines (RL95-2, Ishikawa and Ishikawa 3H12) and an epithelial cell-line (HEK293T) was compared. Next, to test Trib2 importance for embryo implantation in human, we used an *in vitro* binding assay based on a 2D co-culture of endometrial and trophoblast (JAr) cells. Finally, using human 2- and 3-Dimentional cell culture models, we studied the outgrowth of trophoblast spheroids on endometrial (RL95-2 and Ishikawa) and non-endometrial epithelial (HEK293T) cells over the course of 24, 48 and 72 hr.

Results: No embryo successfully implanted in the uterine horns of Trib2 null females indicating the involvement of Trib2 protein in the implantation process. Though, HEK293T cells are from non-reproductive origin, the endogenous expression of both TLR5 and Trib2 proteins in this cell-line, made it the optimum model for inspecting the Trib2 functions in humans. Using the HEK293T cells in the 2D binding assay we showed that the percentage of embryo attachment decreased when Trib2 gene expression was knocked down by siTrib2. Using the same model, we showed p38-MAPK pathway is also negatively modulated by Trib2. Studying JAr spheres outgrowth experiments showed different rates of outgrowth between 2D and 3D culture models. But the rate of spheroids outgrowth on RL95-2 cells was significantly higher compared to Ishikawa and HEK293T cells in both the 2D and the 3D models. Flagellin stimulation of the RL95-2 epithelial cells in both models lowered the rate of spheres outgrowth.

Conclusion: Our results demonstrated that Trib2 is essential for successful embryo implantation in mice. Using a non-reproductive cell-line HEK293T cells, helped us inspect the role of Trib2 in human embryo implantation *in vitro* and showed that Trib2 is involved in embryo implantation since its knockdown significantly reduced the percentage of attached Jar spheroids to the epithelial monolayer. Hence further *in vivo* studies are needed to confirm these results. Endogenous expression of Trib2 in HEK293T cells and the functionality of TLR5 signaling pathway in these cells also made it a suitable model for studying the Trib2 modulation of TLR5 signaling pathway. RL95-2 cells which represented receptive endometrium induced the highest rate of trophoblast outgrowth, indicating that trophoblast proliferation to form connection with the endometrial cells is better supported by a receptive endometrial epithelial cells. We are currently comparing the TLR5 and Trib2 related gene expression profile between the endometrial biopsies of healthy women and IVF-failed patients to further investigate the role of Tribbles proteins in regulation of Human fertility.

Key words: *Toll-Like Receptors, Tribbles-2, Embryo implantation, Female fertility, Endometrial receptivity.*

A-2

Testis tissue engineering: Novel scaffolds composed of human serum albumin for growth of human testicular cells

Borzouie Z[1, 2], Jebali A[3], Naghibzadeh M[1, 2], Pourrajab F[4], Nikukar H[1, 3], Talebi AR[2], Khoradmehr A[2], Sadeghian-Nodoushan F[1], Aflatoonian B[1, 3, 5], Hekmatimoghaddam SH[1, 6].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Biochemistry, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
6. *Department of Laboratory Science, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: b.aflatoonian@ssu.ac.ir; shhekmati2002@yahoo.com

Introduction: Tissue engineering has been considered as an interesting field that use engineering and scientific methods to develop biological substitute for improving or reconstruction of tissue.

Materials and Methods: The hTCs were isolated from three non-obstructive azoospermia TESE samples from the patients attending Yazd Reproductive Sciences Institute, as well as two normal tissues from fertile men undergoing orchidectomy for prostate cancer, all after obtaining signed informed consent. To investigate the presence of spermatogonial cells (SCs) in the seminiferous tubules, immunofluorescent (IF) staining was done using two highly specific markers for SCs; GFRA1 and GPR125. Samples were treated with two steps of enzymatic digestion overnight, followed by culture of single cells in flasks with DMEM +10% fetal bovine serum. The presence of SCs among the hTCs was assessed after 4 passages using IF, and a heterogeneous population of hTCs were plated onto two different scaffolds; 1) new human serum albumin (HSA)/calcium phosphate 3D scaffold, and 2) electrospun polyvinyl alcohol (PVA) /HAS/gelatin nanofibers. Glial cell-derived neurotrophic factor, epidermal growth factor and follicle stimulating factor were added to the culture media. Scanning electron microscopy (SEM) images were taken before and after culture. Cell viability was assessed by MTT assay at days 7 and 14.

Results: IF results showed lack of SCs within the cultured hTCs after 4 passages, although a few SCs had been detected within the TESE samples. MTT and SEM data proved the viability and proliferation of hTCs after plating on the HAS /calcium phosphate 3D scaffolds and also PVA /HAS /gelatin nanofibers, without any significant difference between the two scaffolds. However, it seems objectively that nanofibers have provided a better extra cellular matrix (ECM) to support hTCs in culture.

Conclusion: The two different types of homemade scaffolds satisfactorily supported the ex vivo growth of hTCs. Further modifications may improve these culture devices to be applied in tissue engineering and regenerative medicine in male infertility.

Key words: *Electrospun, Extracellular matrix, Testicular sperm extraction, Human serum albumin, Tissue engineering.*

A-3

Association of APPL1 with insulin and adiponectin receptors in granulosa cells of patients with polycystic ovary syndrome

Dehghan R[1], Saidijam M[1], Mehdizadeh M[2], Najafi R[3], Shabab N[1], Artimani T[3].

1. *Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
2. *Cellular and Molecular Research Center, Faculty of Advanced Technology in Medicine, Anatomy Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
3. *Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.*

Email: artimani@umsha.ac.ir

Introduction: Polycystic ovary syndrome (PCOS) with symptoms such as clinical or biochemical hyperandrogenism, chronic anovulation and polycystic ovaries has been identified and is commonly associated with insulin resistance. Resistance to insulin has been observed at 50-70% of women with PCOS. Insulin resistance in PCOS may be related to the obesity and reduction of adiponectin. APPL1 is the first mediator protein that plays important role in intracellular signal transduction of adiponectin receptors pathway. Recently, experimental evidences demonstrated that knockout (KO) of APPL1 in mice may lead to the reduction of insulin and adiponectin signaling and causes insulin resistance.

Materials and Methods: In this study 44 infertile women 18-40 yr old who underwent oocyte recovery at an IVF clinic were recruited; 22 PCOS patient and 22 infertile women with normal ovulatory function as control group. After approval of Hamadan University of Medical Sciences Ethics Committee and written informed consent of the patients, human granulosa cells were obtained from women undergoing oocyte retrieval and were separated from aspirated follicular fluid. A series of isolation and purification methods were performed including density gradient centrifugation, MACS (use of antibody bead complexes) and RNA extraction. RT-PCR was applied to show the existence of APPL1, insulin and adiponectin receptors in granulosa cells. Quantitative real-time PCR analysis was applied to investigate the relative expression of these genes in purified granulosa cells.

Results: Our result showed that expression of APPL1 significantly reduced in PCOS women with BMI $\leq$30 and BMI $\geq$30 compared to the BMI-matched non-PCOS women. (p=0.04, p=0.02, respectively). Also the expression of INSR significantly diminished in the PCOS women compared to the controls (p=0.04). Morever cellular expression of adiponectin (p=0.001), adipoR1 (p=0.003) and adipoR2 (p=0.02) in PCOS were significantly lower than control group. In obese PCOS women (BMI $\geq$30), adiponectin R1 expression, significantly diminished compared to the BMI-matched non-PCOS women (p=0.02). Based on Spearman test, there were significant positive correlations between

INSR and adipoR1 (r=0.48, p=0.001), INSR and AdipoR2 (r=0.61, p=0.001) and APPL1 and INSR (r=0.48, p=0.001).

Conclusion: Our findings suggest that APPL1 might be as a crucial mediator in adiponectin and insulin signaling in GC and may be as an important factor in development of PCOS and resistance to the insulin.

Key words: *APPL 1, Insulin receptor, Adiponectin receptor, Granulosa cell, Polycystic ovary syndrome.*

A-4

The immunomodulatory effects of decidual microenvironment on dendritic cells

Eskandarian M, Jahangiri AH, Hyaftiyan, A Moazzeni SM.
Departmetn of Immunology, Tarbiat Modares University, Tehran, Iran.
Email: maryamskandaryan@yahoo.com

Introduction: Dendritic cells (DCs) can acquire immunogenic or tolerogenic properties depending on tissue environmental factors and cell- cell interactions. In this study we aimed to determine the immunomodulatory effects of decidual cells from resorbtion and non-resorbtion decidua on DC functions.

Materials and Methods: DCs were differentiated from mouse bone marrow (BM) cells in the presence of DC differentiation cytokines, GM-CSF and IL-4. DCs were co- cultured with the decidual cells from resorbed and non-resorbed fetuses and their immunophenotype was evaluated through flow cytometric analysis. Dextran uptake was also studied for the assessment of phagocytotic ability of the generated DCs.

Results: Our results indicated that treatment of dendritic cells with decidual cells from resorbtion decidua significantly increased MHCII, CD40 and CD86 expression by DCs. Diminished endocytic capacity was also observed in DCs that were treated with resorbtion decidua.

Conclusion: It can be concluded that decidual microenvironment could alter the DCs phenotype and functions through cell- cell interactions and decidual-secreted factors. DCs as regulators of innate and adaptive immune responses could also determine the pattern of immune responses at the feto-maternal interface and, subsequently, pregnancy outcome.

Key words: *Dendritic cells, Decidua, Resorbtion.*

A-5

Spermatogenesis regeneration after grafting neonate mouse testicular tissue into epididymal fat of mature mouse

Eyni H, Mazaheri Z, Movahedin M.
Department of Anatomical Sciences, Tarbiat Modares University, Tehran, Iran.
Email: h.eyni@modares.ac.ir

Introduction: Testicular grafting has the potential to become a method to preserve fertility in prepubertal boys undergoing cancer treatment.

Materials and Methods: Three neonate male mice aged 3-5 days as the donors and three mature male mice aged 6-8 weeks as the recipients were used. After bilaterally castration of recipients, four pieces of donor fragments (approximately 1 mm^3) were grafted into epididymal fat next to the testicular artery. Eight weeks after transplantation, grafted testicular tissue were collected. Hematoxylin and eosin (H&E) staining was used to evaluate germ cell differentiation, immunohistochemistry staining by proliferating cell nuclear antigen antibody, real-time RT-PCR to evaluate and to identify the expression of genes that are involved in spermatogenesis development and TUNEL assay for apoptosis frequency.

Results: Vascular anastomoses were seen at the graft site. At the time of grafting, spermatogonial cells were the only germ cells present in the seminiferous tubules. Eight weeks after transplantation, histological, real-time RT-PCR and immunohistochemical analyses of the grafts showed differentiation up to the spermatid level. TUNEL assay showed no significant difference after transplantation.

Conclusion: The results of previous studies showed arrest of spermatogenesis in meiotic. Due to the appropriate hormonal and temperature conditions of epididymal fat, it seems grafting of neonate testicular tissue to epididymal fat may be a powerful site to recovery of spermatogenesis and may pave the way for fertility preservation among infant patients.

Key words: *Spermatogenesis, Graft, Testis tissue, Epididymal fat.*

A-6

Does rescue oocyte in vitro maturation (IVM) impair embryo morphokinetics development? a time lapse study

Faramarzi A, Khalili MA, Ashourzadeh S, Fesahat F, Agha-Rahimi A.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: faramarzi_90@yahoo.com

Introduction: Rescue in vitro maturation (IVM) of oocytes is not a routine procedure in association with assisted reproductive technique (ART). In addition, best embryo selection using time lapse monitoring (TLM) is an important challenge in ART.

Materials and Methods: Morphokinetic variables (time to 2nd PB extrusion (SPBE), pronuclei (PN) appearance (PNA), PN fading (PNF), time to 2 cells (t2), t3, t4, t5, t6, t7, t8, S1 (t2-PNF), S2 (t4-t3), CC2 (t3-t2), CC3 (t5-t3) and S3 (t8-t5) as well as abnormal cleavage patterns of 150 zygotes derived from IVM oocytes (group I) and 218 zygotes derived from in-vivo matured oocytes (group II) were compared in regard to Zona pellucida (ZP) birefringence and meiotic spindle (MS) visualization with PolScope. Also, CCs expression of apoptotic gene (Bax, Bcl2 and Caspase 3) were quantified using reverse transcription Q-PCR.

Results: Time of SPBE, PNF, t2, S1, t3, t4 and S2 happened later in zygotes derived from IVM oocytes compared to zygotes derived from in vivo matured oocytes (p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, respectively). But, only CC2 occurred earlier in zygotes derived from in-vivo matured high ZP birefringet and MS seen oocytes (p=0.006). The rates of uneven blastomeres, reverse, direct and arbitrary cleavage embryos increased in group I (p=0.005, p=0.001, p=0.002, p=0.001, respectively). Also, apoptotic gene expression increased in CCs group I compared to group II (p>0.05).

Conclusion: Some of morphokietics timing occurred later in zygote derived from IVM oocytes. In addition, abnormal morphokinetics behavior increased in zygotes derived IVM oocytes. There is an increasing trend for CCs apoptotic genes in IVM oocytes. Improvement in IVM culture have been proposed to dominate the spontaneous maturation process that influences subsequent embryo development especially in women with small number of oocytes. In addition, abnormal cleavages pattern are detected by TLM, which make TLM very efficient tool in single embryo transfer (SET) program.

Key words: *Morphokinetics, In vitro maturation, ZP birefringence, Meiotic spindles, Embryos.*

A-7
Vitrification of mouse MII oocytes: Developmental competency using Paclitaxel

Fesahat F[1, 2], Faramarzi A[1], Khoradmehr A[1], Omidi M[1], Anbari F[1], Khalili MA[1].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: farzaneh.fesahat@gmail.com

Introduction: Oocyte cryopreservation provides an important alternative for fertility preservation for women who will be treated with cytotoxic drugs. However, it can cause spindle disorganization of microtubules, putting the zygote at risk for aneuploidy. Paclitaxel is known to stabilize the microtubules that constitute the spindle. The aim of this study was to investigate the suitable concentration of paclitaxel for adding to the vitrification media to improve the developmental potential of post-thawed mature oocytes to blastocyst formation in mice.

Materials and Methods: A total of 300 MII oocytes were retrieved from superovulated mice, and were divided into three groups of control, experimental I, and experimental II. Oocytes in experimental I and experimental II were cryopreserved in the presence of 0.5 µM or 1 µM of paclitaxel in vitrification media, respectively. After thawing, all oocytes were incubated in G-IVF medium for 1 hour. From each group, 12 oocytes were selected for viability evaluation by Hoechst/propidium iodide nuclear staining. Standard in vitro fertilization was performed on the rest of the oocytes and embryo development was followed to the blastocyst stage.

Results: Fertilization rate was not significantly different between the three groups. However, the cleavage rate (55%) in experimental II group was significantly lower compared to experimental I (88%) and control groups (83%). There was a detectable difference between the three groups at the blastocyst rate (experimental I and control groups, p=0.004; experimental II vs. control and experimental I, p<0.001).The highest rates of parthenogenesis and arrest were in experimental II (16% and 21%, respectively) compared with control (6% and 5%, respectively) and experimental I (5% and 3%, respectively). There was also a significant decrease in viability rate of oocytes in experimental II compared to the other groups.

Conclusion: A high concentration of paclitaxel, an anticancer drug, interrupted the mouse oocyte competency when supplemented to vitrification media. Consequently, the optimal concentration of this cytoskeleton stabilizer may improve the post-thawed developmental abilities of oocytes.

Key words: *Embryo development, Mouse, Oocyte viability, Paclitaxel, Vitrification.*

A-8
Epigenetic alterations of *CYP19A1* gene in Cumulus cells and its relevance to infertility in endometriosis

Hosseini E[1, 2], Mehraein F[2], Shahhoseini M[3], Karimian L[4], Ashrafi M[5], Afsharian P[3], Aflatoonian R[5].

1. *Department of Obstetrics and Gynecology, Shahrekord University of Medical Sciences, Shahrekord, Iran.*
2. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
3. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
5. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: elhamhosseinid@gmail.com

Introduction: Endometriosis, the growth of endometrial-like cells outside the uterus, is thought to occur due to differential regulation of gene expression in ectopically growing tissues. 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.

Materials and Methods: Cumulus cells were obtained from 24 infertile patients with and without endometriosis who underwent ovarian stimulation for intracytoplasmic sperm injection. Expression of *CYP19A1* gene was quantified using reverse

transcription Q-PCR. DNA methylation, histone modifications, and binding of Estrogen Receptor, ERβ to regulatory DNA sequences of *CYP19A1* gene were evaluated by Chromatin Immuno Precipitation (ChIP) assay.

Results: *CYP19A1* gene expression in CCs of endometriosis patients was significantly lower than the control group (p=0.04). Higher incorporation of MeCP2 (as a marker of DNA methylation) on PII and PI.4 promoters, and hypoacetylation at H3K9 in PII and hypermethylation at H3K9 in PI.4 were observed in *CYP19A1* gene in endometriosis patients ($p \leq 0.05$). Moreover, a decreased level of ERβ binding to PII and an increased level of its binding to PI.3 and PI.4 promoters of *CYP19A1* were observed in endometriosis patients when compared to control.

Conclusion: Significant reduction of *CYP19A1* gene expression in CCs of endometriosis patients may be the result of epigenetic alterations in its regulatory regions, either by DNA methylation or histone modifications. These epigenetic changes along with differential binding of ERβ (as a transcription factor) in *CYP19A1* promoters may impair follicular steroidogenesis, leading to poor oocyte and embryo condition in endometriosis patients.

Key words: *Endometriosis, Epigenetic, CYP19A1, Cumulus cell, Estrogen receptor beta.*

A-9
Characterization of extracellular vesicles secreted by the primary oviductal epithelial cells

Jamaludin AN[1], Witek KJ[2], Meikle A[1], Hunt S[3], Ebbens S[5], Andrownoska A[2], Thurston L[5], Fazeli A[1, 6].

1. *Academic Unit of Reproductive and Development Medicine, University of Sheffield, Sheffield, UK.*
2. *Institute of the Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland.*
3. *The School of Clinical Dentistry, University of Sheffield, Sheffield, UK.*
4. *Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK.*
5. *Royal Veterinary College, University of London, London, Iran.*
6. *Department of Pathophysiology, University of Tartu, Tartu, Estonia.*

Email: najamaludin1@sheffield.ac.uk

Introduction: The interaction of gametes and embryo with the maternal environment has a crucial impact on gametes maturation, embryonic development and subsequent pregnancy success. Recent studies have recognised extracellular vesicles (EVs) as potent vehicles for intercellular communication. Defining the type of EVs which are produced by different reproductive cells will help us to understand how these structures can influence reproductive processes. The aims of the current investigation are to compare size, concentration and physical properties of EVs secreted by Porcine oviductal epithelial cells (POECs) in vitro in conditioned medium (CM) after 24 and 48 hours of cell culture, as well as comparing EVs secreted by isthmic and ampullar regions of the oviduct.

Materials and Methods: Primary porcine oviductal epithelial cells (POEC), primary porcine isthmus epithelial cells (PIEC) and primary porcine ampulla epithelial cells (PAEC), were cultured *in vitro* in EVs depleted medium. CM were collected after 24 or 48 hours of cell culture for POEC. However, for PIEC and PAEC, CM were collected once the cell reached 70% confluency. EVs were successfully isolated from CM using size exclusion chromatography. Nanoparticle tracking analysis was performed to evaluate EV size range and concentration. Electrical surface properties were determined by zeta potential by Zetaview (Particle Metrix, Meerbusch, Germany).

Results: The concentration of EVs secreted by POEC was time dependent and exhibited significant different time dependant changes in zeta potential values. EVs size distribution was not significantly different between EVs secreted by POECs after 24 or 48 hours of cell culture. EVs secreted by PIEC and PAEC showed no significant difference in concentration and size distribution. However, there was a difference in zeta potential values between EVs secreted by these two different region of the oviduct.

Conclusion: Oviductal epithelial cells secrete EVs *in vitro* and surface characteristics of oviductal EVs in primary culture differ over time and the origin of the EVs in oviduct. Further characterization of EVs will enhance our understanding of intercellular communication within the female reproductive tract.

Key words: *Extracellullar vesicles, Oviduct, Oviduct epithelial cells, Zeta potential.*

A-10
Effect of static magnetic field on vitrification process and transplantation of mouse ovarian tissue

Kazemein Jasemi VS[1], Samadi F[1], Eimani H[2, 3], Hasani S[1], Fathi R[2], Shahverdi AH[2].

1. *Department of Animal and Poultry Physiology, Faculty of Animal Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan, Iran.*
2. *Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Anatomy, Faculty of Medicine, Baqiyatallah University of Medical Sciences,Tehran, Iran.*

Email: eimanih@royaninstitute.org

Introduction: Ovarian cryopreservation and transplantation has emerged as an important method of fertility preservation. Magnetic field enhanced cryopreservation has been considered in recent times as a promising type of ovarian cryopreservation but the effectiveness of the process is still not clear. The aim of this study was to investigate the effects of applying static magnetic field (SMF) during vitrification process and transplantation of mouse ovarian tissue.

Materials and Methods: The study was done in two parts. In the first part of study ovaries of 6-8 weeks-old female NMRI (Naval Medical Research Institute) mice

were divided randomly into 4 groups: Control 1 group; fresh ovaries immediately were allocated for histology evaluation, V1 group; ovaries were vitrified-warmed without exposure to SMF, V2 group; ovaries were vitrified-warmed with exposure to and vitrified S1 group; ovaries were exposed to SMF just in equilibration step. In the second part of study ovaries randomly were divided into 4 groups: FOT group; fresh ovaries were immediately transplanted into testicular tissue, FOT+ group; fresh ovaries were exposed to the SMF for 10 min then were transplanted into the testicular tissue, VOT group; vitrified-warmed ovaries were transplanted into the testicular tissue and VOT+ group; vitrified-warmed ovaries were transplanted into the testicular tissue then transplantation site were exposed to SMF for 10 min.

Results: In first part the results indicated that the highest percentages of morphological intact primordial follicles were seen in vitrified S1 group ($p<0.05$). In terms of ultrastructure, there was no difference between control and vitrified S1 groups. In the second part of study, best angiogenesis was in the group of FOT+ ($p<0.05$). The rate of oocytes reaching MII stage was higher in the FOT+ than in the other experimental groups.

Conclusion: SMF can exert positive effects in improvement of retention of follicles, reducing follicular death, better angiogenesis, maturation, fertilization and embryo development. Testicular tissue as ovarian receptor site has the ability to accept grafts without suppressing the immune system.

Key words: *Angiogenesis, Static magnetic field, Testis, Vitrification.*

A-11

The effect of 24 hours delay in oocyte maturation triggering in IVF/ICSI cycles with antagonist protocol and not-elevated progesterone

Naghshineh E[1], Davar R[2], Neghab N[2].

1. *Department of Obstetrics and Gynecology, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: naghshineh@med.mui.ac.ir

Introduction: The best time of final oocyte maturation triggering in assisted reproduction technology (ART) protocols is unknown. This time always estimated by combined follicular size and blood progesterone level.

Materials and Methods: All patients who were candidate for ART, underwent controlled ovarian hyperstimulation by antagonist protocol. When at least 3 follicles with ≥17 mm diameter were seen by vaginal ultrasonography; blood progesterone level was measured. The patients who had progesterone level ≤1 ng/dl entered the study. The participants' randomizations were done and patients were divided into two groups. In the first group, final oocyte maturation was done by HCG at the same day, but in the second group, this was performed 24 hr later. Oocytes retrieval was done 36 hr after HCG trigger by transvaginal ultrasound guide.

Results: The numbers of retrieved oocytes, mature oocytes (MII), fertilized oocytes (2PN), embryos formation and transferred embryos and the embryos quality have not significant differences between two groups ($p>0.05$). Also, fertilization and implantation rate, chemical and clinical pregnancy did not differ between groups.

Conclusion: Delaying of triggering oocyte maturation by 24 hours in antagonist protocol with not-elevated progesterone (progesterone ≤1 ng/ml) have not beneficial nor harmful effect on the number of mature oocytes (MII) and other IVF cycle characteristics.

Key words: *Oocyte maturation triggering, ART, IVF results.*

A-12

Pregnancy outcomes in women with history of repeated implantation failure after intrauterine infusion of autologous platelet-rich plasma (PRP) in frozen-thawed cycles

Neghab N[1], Eftekhar M[1], Naghshineh E[2], Khani P[1].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Obstetrics and Gynecology, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: nosrat20@yahoo.com

Introduction: Recently, intrauterine infusion of platelet-rich plasma (PRP) is described to promote endometrial growth and receptivity. PRP is prepared from fresh whole blood that contained several growth factors and cytokines including fibroblast growth factor (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VGEF), transforming growth factor (TGF), insulin-like growth factor I, II (IGF-I, II), connective tissue growth factor (CTGF) and interleukin 8 (IL-8).

Materials and Methods: In this clinical trial 33 women with a history of 2 or more implantation failure who were candidates for frozen-thawed embryo transfer were recruited in this study. Intrauterine infusion of 1 ml of platelet-rich plasma that contained platelet 5-6 times more than peripheral blood sample was performed 48 hrs before cleavage transfer. In control group 33 women received routine medication for frozen thawed cycle.

Results: chemical, clinical and ongoing pregnancy were higher in PRP group(36.4% vs 24.2%, 33.3% vs 24.2%, 24.2% vs 18.2%) but these results were not significant ($p=0.422$, 0.587, 0.764 respectively).

Conclusion: It seems that platelet-rich plasma may be effective in improvement of pregnancy outcome in patients with history of implantation failure.

Key words: *Platelet-rich plasma, In Vitro Fertilization, Pregnancy rate.*

A-13
Molecular and functional effects of Melatonin on PCOS oocyte maturation

Nikmard F[1], Bakhtiyari M[1], Hosseini E[1], Ashrafi M[2], Amidi F[3], Aflatoonian R[2].
1. *Department of Anatomy, Iran University of Medical Sciences, Tehran, Iran.*
2. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Aanatomy Department, Tehran University of Medical Sciences, Tehran, Iran.*

Email: nikmardf@gmail.com

Introduction: The purpose of the in vitro maturation of oocytes is generation of mature oocyte that are capable of supporting future development. Efforts to enhance oocyte developmental competence by developing optimal culture conditions have been met. Although melatonin as a free radicals scavenger has been shown to exhibit genomic actions, regulates the antioxidant genes expression and apoptosis mechanisms. However, little information is available the effect of melatonin on expression of genes involved in oocyte maturation.
Materials and Methods: Seventy-seven female prepubertal (21-25 day-old) C57BL/6 mice were purchased from Pasteur Institute of Iran. The animals were housed in a temperature-controlled environment. The mice were randomly divided into two groups: PCOS model group injected with (s.c 6 mg/100 g body weight) dehydroepiandrosterone, dissolved in 0.01 mL 95% ethanol and mixed with 0.09 mL olive oil, for 20 consecutive days and the control group injected with (s.c) 0.09 mL olive oil and 0.01 mL 95% ethanol daily for 20 consecutive days. After IVM, pools of mature oocytes in different concentration of melatonin from both of PCOS model and control group were separately analyzed by qPCR. GDF9, BMP15, antioxidants and apoptosis genes expression were analyzed.
Results: Melatonin improved the maturation that significant maturation of PCOS oocytes (81.1% vs. 56.3%, $p<0.05$) were achieved with 10^{-6} M concentration. Cleavage rate after in vitro fertilization of these oocyte was significantly different with 10^{-5} M concentration in PCOS oocyte (54% vs. 35%) and with 10^{-6} M concentration in control group (55% vs. 38%). In this study, it is demonstrated that melatonin influences the expression of GDF9 and BMP15 genes in PCOS oocytes and it can provide valuable support for the ability to protect the developmental potential during the in-vitro maturation process. Furthermore, melatonin was increased antioxidants genes expression and regulates apoptosis pathway in PCOS oocyte so it effectively reduces the adverse effects of medium culture conditions on PCOS oocyte.
Conclusion: Current investigation showed that melatonin can induce oocyte maturation and guarantee oocyte developing potential. These findings demonstrated that the high concentrations of melatonin in the medium culture can serve to protect the PCOS oocytes from toxic oxygen products. In general, the molecular effect of melatonin was dose-dependent and high concentration of melatonin can improve the quality of the PCOS similar to the control oocyte.
Key words: *In-vitro maturation, Melatonin, Developmental potential.*

A-14
Sperm DNA, chromatin and acrosome integrity in vitrification vs. solid surface or vapor

Rahiminia T, Talebi AR, Hosseini A, Ebrahimian N, Ghasemi-Esmailabad S.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: tahereh.rahiminia@gmail.com

Introduction: Presence of vitrification method in sperm freezing and introduction of solid surface vitrification beside rapid freezing in vapor consider to help infertility centers. While the effects of cryopreservation on motility, morphology and viability of sperm are documented, the question of the probable alteration of sperm DNA, chromatin and acrosome integrity after freezing and thawing procedures in different methods is still controversial.
Materials and Methods: Normal sample were collected according WHO strict criteria. Sperm suspensions were mixed 1: 1 with 0.5 M sucrose and divided into four equal aliquots for freezing: fresh, nitrogen direct immersion vitrification (Vit), solid surface vitrification (SSV) and in vapor (Vapor). Sperm suspensions were transferred into a 0.25 ml sterile plastic. Then straw was inserted inside the 0.5 ml straw. For thawing, the straws were immersed in a 42°C water bath. Beside sperm parameters, we assessed the acrosome reaction by double staining, chromatin integrity by toluidine blue (Tb) and chromomycin A3 (CMA3) and DNA integrity by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) respectively.
Results: In progressive motility, the highest rate was happened in Vit (39.9±13.3). Moreover, the lowest rate of immotile sperm was in Vit (32.7±16.3). In normal morphology, Vit group was similar to the fresh, while SSV and Vapor were significantly different from the fresh. The percentage of acrosome reacted sperms was more in Vit (81.3±10.2) than fresh group. TUNEL+ results shows that DNA fragmentation was significantly increased in Vit ($p=0.025$). While in SSV and Vapor results were comparable to fresh. There was a significant correlation between TUNEL+ and normal morphology, TB, CMA3 and presence of intact acrosome.
Conclusion: Sperm in Vapor was healthier in terms of DNA, chromatin and acrosome integrity. In contrast of motility and morphology retention, DNA, chromatin and acrosome integrity was decreased in Vit. However these findings were better in SSV or Vapor.
Key words: *Solid surface vitrification, Vapor, DNA integrity, Chromatin integrity, Acrosome integrity.*

Oral Presentations

7th Yazd International Congress and Student Award in Reproductive Medicine

O-1
Effects of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: A pilot study

Nazari L, Salehpour S, Hoseini S, Zadehmodarres Sh, Ajori L.
Department of Obstetrics and Gynecology, Preventive Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: nazari@sbmu.ac.ir

Introduction: Repeated implantation failure (RIF) is a major challenge in reproductive medicine and despite several methods that have been described for management, there is little consensus on the most effective one.
Materials and Methods: Twenty women with a history of RIF who were candidates for frozen-thawed embryo transfer were recruited in this study. Intrauterine infusion of 0.5 ml of platelet-rich plasma that contained platelet 4-5 times more than peripheral blood sample was performed 48 hrs before blastocyst transfer.
Results: Eighteen participants were pregnant with one early miscarriage and one molar pregnancy. Sixteen clinical pregnancies were recorded and their pregnancies are ongoing.
Conclusion: According to this study, it seems that platelet-rich plasma is effective in improvement of pregnancy outcome in RIF patients.
Key words: *Fertilization in Vitro, Implantation, Platelet-rich plasma, Pregnancy rate, Repeated implantation failure.*

O-2
Combined genomic and proteomic analysis of endometrium in women with polycystic ovarian syndrome compared to normal

Amjadi FS[1], Alikhani M[2], Ashrafi M[3], Mirzaei M[4], Mehdizadeh M[5], Hosseini Salekdeh Gh[2, 6], Aflatoonian R[7].
1. *Department of Anatomy, School of Medicine, Iran University of Medical Science, Tehran, Iran.*
2. *Department of Molecular Systems Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
3. *Obstetrics and Gynecology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
4. *Department of Chemistry and Bimolecular Sciences, Macquarie University, North Ryde, NSW, Australia.*
5. *Cellular and Molecular Research Center, Department of Anatomy, Iran University of Medical Sciences, Tehran, Iran.*
6. *Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran.*
7. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: amjadi.bh@gmail.com; r.aflatoonian@gmail.com

Introduction: Growing evidence suggests that a disorder of endometrial receptivity may contribute to the adverse reproductive outcomes in polycystic ovarian syndrome (PCOs). PCOs is a complex disease causing infertility in about 6-15% of reproductive-age women.
Materials and Methods: Total mRNA and protein were extracted from endometrial tissues of PCOS patients (n=6) and healthy fertile individuals (n=6) during luteal phase, then analyzed using qRT-PCR array and shotgun proteomics approach, respectively. To validate this investigation western blot and quantitative real time PCR were performed.
Results: mRNA evaluation showed significant over-expression of the genes which are involved in carcinoma, coagulation and cytoskeleton and downregulation of cell adhesion molecules in endometrial samples of PCOs women. Shotgun proteomics analysis allowed the identification of beyond 995 proteins, of which 150 proteins showed more abundance, and 46 proteins showed less abundance in PCOs. The negatively altered proteins were categorized in biological processes such as cytoskeleton organization, blood coagulation, and mitotic cell cycle. The results obtained in the western blot and real time PCR followed a similar regulation of proteomic analysis.
Conclusion: This study provide the first insight into the combined global protein and gene expression in the endometrium of PCOS patients which affected endometrial receptivity. There is a lack of correlation between endometrial proteomic data with gene expression findings in women with PCOs, maybe due to post-transcriptional or translational regulation. However, the alteration of genes related to cytoskeleton and blood coagulation detected by PCR array was also supported by our protein results. Each of them absolutely demonstrates an important role in endometrial receptivity. Genomic analysis has also shown upregulation of some tumor markers in endometrium of PCOs women which may explain the increased risk of endometrial carcinoma in these patients.
Key words: *Endometrium, Proteomics, Genomic, PCOs, Luteal phase.*

O-3
Toll-like Receptor 9 activation in trophoblast-endometrium cross-talk

Maslehat Lay N[2], Sanchez-Lopez J[2], Montazeri M[2], Caballero I[2, 3], Elliott S[2], Fazeli A[1, 2].
1. *Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu, Tartu, Estonia.*

2. *Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.*
3. *UMR1282 ISP, INRA, Nouzilly, France.*
Email: nasimmaslehat@yahoo.com

Introduction: Implantation failure caused by sexually transmitted infections (STI) is one of the major factors involved in pregnancy loss. Successful implantation requires a supportive environment, which is strongly dependent on a healthy endometrium. Presence of any infection at the site of the implantation could be sensed by pathogen recognition receptors (PRRs). Toll-like receptors (TLRs) are a major family of PRRs that are widely expressed in endometrial epithelial cells and react to specific microbial agents. These receptors initiate intracellular signaling, leading to secretion of inflammatory cytokines that might prevent implantation. The aim of the current investigation was to study TLR9 activation via its specific ligand (CpG) in human endometrial epithelial cells and its effect on trophoblast behavior.
Materials and Methods: An *in vitro* co-culture system of RL95-2- a human endometrial epithelial cell line- and multi-cellular spheroids of JAr cells -a choriocarcinoma cell line- were used to simulate the early stage of human implantation. A stable TLR9 knocked-down RL95-2 cell line was generated using TLR9 specific siRNA in order to determine whether TLR9-mediated attachment impairment was from endometrial or trophoblast origin. Wild-type (WT) and TLR9 knocked-down (KD) RL95-2 cells were stimulated with 0, 0.01, 0.1 and 1μM CpG for 24 hours before co-incubation with JAr spheroids and the number of attached spheroids was determined.
Results: The results indicated that stimulation of TLR9 in WT RL95-2 cells with different concentrations of CpG led to a reduction of the percentage of trophoblasts attached to the endometrium in a dose-dependent manner. This inhibitory effect was seen as soon as 4 hours after TLR9 activation. Application of specific TLR9 antagonist to WT RL95-2 cells was able to restore the trophoblast attachment to the endometrium. Attachment of JAr spheroids to KD RL95-2 cells was also impaired when CpG was kept in the co-culture media in contact with the JAr cells. However, when CpG was washed away from pre-treated KD RL95-2 cells before co-culture with the JAr spheres, no difference in trophoblast attachment between controls and CpG-treated KD RL95-2 cells was observed. Similarly, the blockage of TLR9 in the JAr spheroids with a specific antagonist before co-culturing with CpG-treated KD RL95-2 cells did restore trophoblasts implantation to the endometrial cell line. These findings indicate that the effect of TLR9 signaling on implantation failure was originated from both endometrial epithelial cells and trophoblasts.
Conclusion: To conclude, activation of TLR9 negatively affected the JAr spheroids interaction with the endometrial epithelial cells. This could be a potential cause of early implantation failure in infertile couples.
Key words: *Toll-like receptors, Implantation, Intracellular signaling, CpG.*

O-4
Role of epigenetic modification of *HOX* genes in etiology of endometriosis

Golestan Jahromi M[1], Aflatoonian R[2], Afsharian P[3], Barzegar M[3], Shahhoseini M[3], Aflatoonian A[1].
1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive, Tehran, Iran.*
3. *Department of Genetics, Royan Institute for Reproductive Biomedicine, Reproductive Biomedicine Research Center, ACECR, Tehran, Iran.*
Email: golestanm48@yahoo.com; marsha244@yahoo.com

Introduction: Endometriosis, characterized by the presence and growth of functional endometrial-like tissues outside the uterine cavity, is a common and benign gynecological disorder. It has been regarded as a hormonal, immunological, environmental (pollution and toxins) and genetics disease. But the novel hypothesis is an epigenetic modification for enigmatic etiology and pathophysiology of endometriosis. The current study was designed to investigate epigenetic modification (DNA methylation) of promoter region of HOX family genes.
Materials and Methods: Samples obtained from fifteen patients with endometriosis in the reproductive age with normal menstrual cycles, where the same patient provided both eutopic and ectopic endometrium (endometriomas) and 15 cases without endometriosis as control whose samples were surgically checked for the absence of endometriosis. Epigenetic modification (DNA methylation) of 84 HOX genes related family assessed using MeCP2 antibody and Chip qPCR Arrays technique. Informed consent was obtained from patients. All measurements were performed in triplicates on independent biological replicates.
Results: Our data showed significant hypermethylation or hypomethylation of 63 genes of 84 HOX genes in euotopic and ectopic tissue versus control group. Our data showed hypo-methylation of genes that have positive role in cell migration, cell invasion (LBX1, ALX4, HOXD3 and TLX1), cellular proliferation (TLX1, NKX3.1, CDX2, PITX2, SIX6), angiogenesis (ISL-1, PHOX2-B, HOXD3, ISL-2 and HHEX), tumorigenesis (ALX4 and LBX1) and pain generation (HOXC8, LMX1B, PAX3, PITX3, SIX3, SIX6, EN1, EN2, ISL1, HOXD1, SHOX2 and LBX1) in ectopic and in some cases euotopic tissues compare to control group. But our finding indicated hyper- methylation of promoter of other genes that have opposite effect compare to first group genes: negative role in cell migration, cell invasion (MSX2, SIX1, MSX1 and PITX1), cellular proliferation (PITX1), angiogenesis (MSX2 and MSX1) and negative role in tumorigenesis (HOXB13, MSX1) in ectopic and in some cases euotopic groups compare to control group.
Conclusion: Aberration methylation in HOX genes promoter especially genes which are involved in various

aspect of endometriosis development, including cell proliferation, invasiveness and progression, may change in expression of these genes and lead to establishment of endometriosis implants. So this study confirms role of epigenetic modification in etiology of endometriosis and various aspect such as recurrency and hypersensitivity.

Key words: *HOX genes, Epigenetic modification, Endometriosis.*

O-5
New approach aiming at improving chances for successful IVF

Lazurenko VV, Muryzina IY, Litvinova KO.
Kharkiv National Medical University, Department of Obstetrics and Gynaecology No 1, Ukraine.
Email: ktrn.a@icloud.com

Introduction: Since 1977 when the first baby was born, thankfully to IVF, great achievements have been made in this field. Although they have granted many couples, whose causes of infertility regarded as insurmountable just decade ago, with hope to have their own child, real number of successful attempts keeps falling behind expected optimistic rate. To tell the truth, it is consistent with real fecundity of healthy uncompromised couple in their best reproductive ages in each particular cycle: around 25%. That is why in order to achieve more successful rate of IVF it is crucially important to deepen our knowledge about tiny ruling mechanisms of implantation. The pivotal significance of micro-environmental interrelationships driven by hormonal regulation cannot be overestimated. That explains why reclamation of the "field" for implantation is so difficult: like to disentangle a poorly intricate hank of yarn. In our study we have made an attempt to improve chances for successful IVF by prescribing cryopreserved placental extract (CPE).

Materials and Methods: There were 120 women under surveillance. The main group comprised 90 women who had been scheduled for IVF program. They were divided into two groups (picked up randomly): 45 patients were prescribed CPE (intramuscularly 1.8 ml at 10, 12, 14, 16, 18 days of menstrual cycle) additionally to routine management (I group), other 45 ones were managed according to conventional guidelines (II group). Otherwise both groups matched to each other. 30 healthy women with uneventful past medical history and no signs suggestive of infertility were chosen as group for control (III). Vascular endothelial growth factor (VEGF) in serum was assessed by immunoenzyme approuch of "sandwich" type (kit of "Вектор Бест", Russian Federation), serum endothelin-1-kit «Endothelin-1», produced by «Amersham pharmacia biotech», UK, serum Glycodelin-A- ELISA kit, IL-1β, IL-2, IL-6, IL-8 and TNFα «Protein profile» (St-Petersburg, RF).

Results: Dynamic relationship between embryo and endometrium starts being established as long as both of them are at the consistent stage of maturity- so called "temporary fertile window". Immunosuppressive activity of Glycodelin secreted into uterine luminal cavity contributes to protection of the embryonic semiallograft at the fetomaternal interface. At the 22nd day of cycle serum Glycodelin level was significantly higher in the I group (9787.3±2325.7 ng/ml) than in the II group (3535.4±2132.6 ng/ml, $p<0.05$). The site of ongoing implantation breaking out with production of numerous growth factors prompts angiogenesis limited to that interface. VEGF is one of main inductor of angiogenesis. Our study elicited that in the cases of IVF pregnancy (II group) VEGF had been significantly higher (346.25±37.31 pg/ml) than in natural conception (28.46±5.61 pg/ml, $p<0.05$). Than VEGF exhibited the trend towards lowering but it remained quite high (208.96±17.81 pg/ml). In the I group where treatment had included CPE, VEGF was insignificantly higher than the level of control group (54.31±6.52 pg/l, $p>0.05$). Also initially both groups preparing to IVF showed overproduction of endothelin-1 (16.5±2.3 ng/ml) compartively to control group value (1.4±0.5 ng/ml, $p<0.05$). After the treatment endothelin-1 decline in the I group was more tangible (2.6±0.7 ng/ml, $p>0.05$ comparatively to the control group) than in the II group (10.9±2.6 ng/ml, $p<0.05$ to the control group value). The study revealed distortion in the local cytokine balance in patients who had been scheduled for IVF pregnancy. Inherent to healthy woman of her reproductive ages Th2-cytokine balance is superseded by Th1-cytokine preponderance with increased values of IL-1β (65.6±2.1 pg/ml), IL-2 (6.7±0.6 pg/ml), IL-6 (26.7±2.9 pg/ml) and TNF-α (58.4±2.8 pg/ml) comparatively to the control group ($p<0.05$). After the treatment with abovementioned approach (I group) patients showed fast decline almost to the level of control group: IL-1β- 41.2±2.4 pg/ml, IL-6 -15.8±1.3 pg/ml, TNFα- 33.8±3.1 pg/ml ($p<0.05$). Obviously pro-inflammatory microenvironment and significant reduction of glycodelin production are the links of vicious circle affecting adversely mechanism of implantation, vascularization building, and transmission to Th2-profile, which prevents from egg-rejection. CPE proved to have benevolent influence on the immunological derangement conducing glycodelin production and drift of endometrial microenvironment towards Th2-cytokine predominance.

Conclusion: Proposed treatment facilitates recovery of reproductive function and increases the likelihood of successful conception and uneventful course of pregnancy.

Key words*: IVF, Glycodelin, Cell therapy.*

O-6
Effects of human amniotic epithelial cells on Naïve CD4+, CD25¬- T cells from unexplained recurrent spontaneous abortion patients

Motedayyen H[1], Tajik N[2], Rezaei A[1].

1. *Department of Immunology, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran.*

Email: hm.k_65@yahoo.com

Introduction: Unexplained recurrent spontaneous abortion (URSA) is a common disorder in 1-5% of women of reproductive age. Several treatments have been used for URSA. However, those are all controversial. Human amniotic epithelial cells (hAECs) have immunomodulatory properties.

Materials and Methods: Naïve CD4+, CD25- T cells isolated from 10 patients with URSA using MACS technique. hAECs were separated from amnion delivered by healthy women with a normal singleton pregnancy. Naïve T cells (4×10^5) were co-cultured at different ratios with hAECs (1:1, 1:2, 1:5, 1:10) along with the positive control (1:0) for 3 days and 6 days. Proliferation of CSFE-labeled naive T cells was stimulated by anti CD3/CD28 (1 µg/ml) and assessed by flow cytometry.

Results: Co-cultured naïve T cells proliferation at 1:2 ratio for 3 days was significantly lower than positive control ($p\leq0.0001$). Although Naïve T cells proliferation at 1:1, 1:5, 1:10 ratios for 3 days was decreased compared to positive control; this decline was not statistically significant. The proliferation of naïve T cells at 1:1, 1:2, 1:5 ratios for 6 days were significantly decreased in compared to positive control ($p\leq0.007$), whereas this decrease at 1:10 ratio was not statistically significant.

Conclusion: These findings suggest that hAECs have suppressive activities on naïve T cells proliferation from URSA patients in vitro. These suppressive effects were time-dependant and often perform through releasing suppressive mediators. Thus, it seems that hAECs may be suitable cell source as therapy for URSA.

Key words: *Amniotic epithelial cells, Naïve T cells, Recurrent spontaneous abortion, Immunomodulatory effects.*

O-7

Placental kisspeptins differentially modulate vital parameters of estrogen receptor- positive and -negative breast cancer cells

Rasoulzadeh Z[1, 2], Ghods R[3, 4], Kazemi T[1, 2], Mirzadegan E[5], Ghaffari-Tabrizi-Wizsy N[6], Rezania S[7], Kazemnejad S[8], Arefi S[8], Ghasemi J[9], Vafaei S[9], Mahmoudi AR[10], Zarnani AH[9, 11].

1. *Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, IUMS, Tehran, Iran.*
4. *Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran.*
5. *Immunobiology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
6. *SFL Chicken CAM Lab, Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, 8010, Austria.*
7. *Institute of Biophysics, Medical University of Graz, Graz, 8010, Austria.*
8. *Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
9. *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
10. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
11. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran.*

Email: Zr.rasoolzadeh@yahoo.com

Introduction: Kisspeptins (KPs) are major regulators of trophoblast and cancer invasion. Thus far, limited and conflicting data are available on KP-mediated modulation of breast cancer (BC) metastasis; mostly based on synthetic KP-10, the most active fragment of KP.

Materials and Methods: From eleven healthy women (22-32 yr) with uncomplicated term pregnancies undergoing elective cesarean, placentas were obtained. Expression of KPs in placental tissues and isolated cytotrophoblast cells was performed by Immunofluorescent staining and Western blotting (WB). In order to assess whether or not KPs are released in soluble form, villous tissues were explanted in plates coated with matrigel and the prescence of KPs in placental explant culture supernatants was investigated by WB and immunoprecipitation using Tosylactivated Dynabeads. In the next step, functional effects of term placental KPs on proliferation, adhesion, Matrigel invasion, motility, MMP activity and pro-inflammatory cytokine production in MDA-MB-231 (estrogen receptor-negative) and MCF-7 (estrogen receptor-positive) cells were surveyed.

Results: KPs were expressed at high level by term placental syncytiotrophoblasts and released in soluble form. Placental explant conditioned medium containing KPs (CM) significantly reduced proliferation of both cell types compared to CM without (w/o) KP (CM-w/o KP) in a dose- and time-dependent manner. In MDA-MB-231 cells, placental KPs significantly reduced adhesive properties, while increased MMP9 and MMP2 activity and stimulated invasion. Increased invasiveness of MDA-MB-231 cells after CM treatment was inhibited by KP receptor antagonist, P-234. CM significantly reduced motility of MCF-7 cells at all time points (2-30 hr), while it stimulated motility of MDA-MB-231 cells. These effects were reversed by P-234. Co-treatment with selective ER modulators, Tamoxifen and Raloxifene, inhibited the effect of CM on motility of MCF-7 cells. The level of IL-6 in supernatant of MCF-7 cells treated with CM was higher compared to those treated with CM-w/o KP. Both cell types produced more IL-8 after treatment with CM compared to those treated with CM-w/o KP.

Conclusion: Taken together, our observations suggest that placental KPs differentially modulate vital parameters of estrogen receptor-positive and -negative BC cells possibly through modulation of pro-inflammatory cytokine production.

Key words: *Placenta, Kisspeptin, Human breast cancer cells, Invasion, Proliferation.*

O-8

Study of Tnp1 and Tekt1 genes expression during in vitro spermatogenesis enhancement in neonatal mouse testis after three dimensional culture

Alrahel A[1], Movahedin M[1], Mazaheri Z[1], Amidi F[2].

1. *Department of Anatomical Sciences, Tarbiat Modares University, Faculty of Medical Sciences, Tehran, Iran.*
2. *Department of Anatomical Sciences, Tehran University of Medical Sciences, Faculty of Medical Sciences, Tehran, Iran.*

Email: Movahed.m@modares.ac.ir

Introduction: Chemo- and radiotherapeutics treatments used for childhood cancer therapy can irreversibly affect fertility in adulthood. In vitro spermatogenesis enhancement in testis tissue has the potential to become a method to preserve fertility in this people. In this study, spermatogenesis enhancement development was evaluated in neonatal mouse testis after three dimensional cultures to understand of spermatogenesis process in molecular level. Tnp1 as a post-meiotic gene can be expressed during in vitro spermatogenesis enhancement in neonatal mouse testis after three dimensional culture.

Materials and Methods: Testis of 10 mouse pup was removed. The size of the pieces was arbitrary, approximately 1 mg in weight or 1 mm3 in size when compacted. One to three testis tissue fragments were transferred to the agarose hexahedrons, the medium was enhanced with growth factors (GDNF, bFGF, EGF, LIF, beta estradiol and progesterone). Eight weeks after three dimensional culture testicular tissues was collected. Total RNA was extracted from the 8 wk 3D cultured tissue of neonatal mouse. The cDNAs were synthesized. For PCR reactions, the target genes (TnP1 and Tekt1) were normalized to a reference gene and calibrated to an adult or neonatal testis.

Results: At the time of three dimensional cultures, spermatogonial cells were the only germ cells present in the seminiferous tubules. Histological study showed only different types of spermatocytes and post-meiotic stages of germ cells could not be detected. The results showed that expression of Tekt1 as a mitotic gene decreased significantly comparing to adult mouse testis (control group) ($p \leq 0.05$). Meanwhile expression of Tnp1, as meiotic gene, increased significantly comparing with neonate mouse testis in beginning of culture ($p \leq 0.05$).

Conclusion: This kind of three dimensional cultures can induce expression of post-meiotic gene, Tnp1, but it remains in molecular level and could not pass beyond meiosis. If we use immunohistochemical techniques, results may be better approved.

Key words: *In vitro spermatogenesis, Three dimensional tissue culture, Post-meiotic gene, Preserve fertility.*

O-9

Human embryonic stem cell-like cells from in vitro embryo twinning blastocysts

Omidi M[1], Aflatoonian B[2, 3, 4], Khalili MA[1].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
2. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
3. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: omidi.marjan@ymail.com

Introduction: Human embryonic stem cells (hESCs) which are derived from pre-implantation embryos are pluripotent with unlimited self-renewal capacity. The potential to form cells from all three germ layer and also germ cells made them important in this area. One of the challenges in hESC derivation is the number of embryos which are donated for research. In vitro embryo splitting can be used to increase the number of the human embryos for the generation of the hESCs in parallel with infertility treatments.

Materials and Methods: Totally, 17 chromosomally abnormal (3PN) embryos were donated to this research after fully ethical consent by the couples attending for the infertility treatment. Following embryo splitting in day 3 from 6-10 cells cleavage embryos, using biopsy pipettes and micromanipulation technique, the whole blastocyst was recovered from the zona pellucida (ZP). The zona-free whole blastocysts which were resulted from embryo splitting were plated onto mitotically inactivated human foreskin fibroblasts (HFF) feeder layers in microdrops.

Results: From 17 donated cleavage embryos, 34 twin embryos obtained which 20 of them were developed to the blastocyst stage. After three to five days of blastocyst culture onto HFF feeder layers, the hESC-like outgrowths were passaged onto new feeder in microdoprs. The initial outgrowths were very similar to hESCs outgrowth; but, after five passages cells were differentiated and further expansion was not succeeded.

Conclusion: In vitro embryo splitting for increasing the number of the human embryos can be used in the future to reserve pluripotent stem cells for the next generations. The challenge still remains to optimize the methods.

Key words: *Blastomere biopsy, Derivation, Human embryonic stem cells, Human foreskin fibroblasts, In Vitro embryo splitting, Microdrop.*

O-10

The peritoneal membrane as a biomaterial scaffold for reproductive tissue engineering

Sarabadani M, Fathi R.

Department of Embryology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: flower201415@gmail.com

Introduction: Peritoneum is a mesothelial layer; that high concentration of growth and hemostasis factors in its extracellular matrix is unique feature for using it as a

biologic scaffold. This membrane has the same embryologic origin as blood vessel and gonad epithelium.

Materials and Methods: In order to prepare decellularized peritoneum, mouse intestinal mesentery was cut and washed in PBS and decellularized with Tris base and EDTA and subsequently ribonuclease and deoxyribonuclease. Then the primary follicles (diameter: 90-110 µm) isolated from the ovarian tissue was divided to two groups: in the control group, follicles were cultured on base medium (α-MEM +10% FBS +1% FSH +1% ITS), and in the experimental group follicles were cultured on decellularized peritoneum with the base medium. After evaluating the decellularization process using specific staining and SEM, the cultured follicles morphology was evaluated after 9 days.

Results: In histological assessments the absence of cell nucleus represents well elimination of the cells and reservation of essential fibers in the tissue. In addition, morphological studies showed increased follicular growth in the experimental group compared to the control group.

Conclusion: Following our data, it was demonstrated that the present protocol is safe and applicable for decellularization of mouse peritoneum to obtain a natural biologic scaffold for tissue engineering with maximum preservation of the three-dimensional structure of extracellular matrix; and peritoneal tissue can play an effective role in improving the development of in vitro follicle culture. But there seems to be a greater need to study improved methods of culture.

Key words: *Decellularization, Tissue engineering, Peritoneum, Intestinal mesentery, Reproductive biology.*

O-11

In vitro derivation of male germ cells from murine bone morrow mesenchymal stem cells through bone morphogenic protein-4 and retinoic acid induction

Behzadi Fard S[1], Mazaheri Z[1], Ghorbanmehr N[2], Movahedin M[1].

1. *Department of Anatomical Sciences, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran.*
2. *Biotechnology Departmnet, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.*

Email: sababehzadi8@gmail.com

Introduction: Recent studies have demonstrated that mesenchymal stem cells (MSCs) have the capacity to differentiate into germline cells under appropriate in vitro and in vivo conditions.

Materials and Methods: Fourth passage of mBMSCs were differentiated to primordial germ like cells (PGC-LCs) and spermatogonial stem like cells (SSC-LCs) by treatment with 25 ng/ml bone morohogenic protein-4 (BMP4) for 4 days and then, by inducer cocktails including retinoic acid (RA), leukemia inhibitory factor (LIF) and basic fibroblast growth factor for 14 days, respectively. Expression of pluripotency (*Pou5F1*, *Nanog*, *c-Myc*) and specific germ cell (*Mvh*, *Piwil2* and *Stra-8*) genes and Pou5F1, Mvh and Stra8 proteins in each stages were analyzed by real time PCR and immunocytochemistry techniques.

Results: The outcomes of qPCR showed that expression of pluripotent genes were significantly increased ($p<0.05$) in initial differentiation process. BMP4 and RA treatment upregulated the expressions of Mvh and Stra-8, respectively. Also c-Myc as an oncogenic gene had significant decrease in the end of experiment comparing to initial phase of differentiation.

Conclusion: Our results showed that mBMSCs can differentiate to PGC-LCs and SSC-LCs by BMP4 and RA treatment. A sequential method for induction of male germ cell can be used as a suitable method for in vitro infertility treatment.

Key words: *Transdifferentiation, Stem cell, Germ cell, Retinoic acid.*

O-12

Oocyte-like cells induction of mouse parietal peritoneum mesothelial stem cells in vitro

Mirzaeiyan L[1], Fathi R[1], Rezazadeh Volojerdi M[1], Baharvand H[2], Esfandiari F[2], Eivazkhani F[1].

1. *Department of Embryology, Reproductive Biomedicine Research Center, Rayan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Stem Cells and Developmental Biology, Cell Science Research Center, Rayan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*

Email: lmirzaey64@gmail.com

Introduction: Parietal peritoneum mesothelial stem cells have been reported to reside in the monolayer of anterior abdominal wall.

Materials and Methods: Direct explants of mouse anterior abdominal peritoneal mesothelium (mAPM) have been used as the source of stem cells. The mAPM-derived stem cells were first isolated then cultured in differentiation medium containing 10% human follicular fluid for 21 days in vitro. Then mAPM-derived stem cells were assessed for expression of PGC markers; Dead (Asp-Glu-Ala-Asp) box polypeptide 4 (Ddx4) and Deleted in azoospermia like (Dazl) and oocyte specific mrkers; Growth differentiation factor-9 (Gdf9), and Zona pellucida glycoprotein 3 (Zp3). The pertinent markers were assessed by immunocytofluorescence.

Results: Our results demonstrated that mAPM-derived stem cells form oocyte-like cells that express oocyte specific markers. Also, cells expressing germ cell markers were observed among these cells.

Conclusion: This study indicated that 10% human follicular fluid can promote the development of oocyte-like cells structures derived from mAPM-derived stem cells.

Key words: *mAPM-derived stem cells, Human follicular fluid, Oocyte-like cells.*

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O-13
46,XX male sex-reversal: Rare condition of developmental sexual disorders

Rasti A[1], Sabbagh-Nejad Yazd S[1], Dehghani MR[2], Kalantar SM[1].
1. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Medical Genetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: rasti_azam@yahoo.com

Introduction: In human, SRY is the Y-chromosomal gene that acts as a trigger for male development. In the absence of a Y chromosome, gonads differentiate into ovaries and female development. 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. 46,XX males can be classified into two subgroups, *SRY*-positive and *SRY*-negative, according to the presence or absence of the *SRY* gene.
Materials and Methods: Two men from the same family and a newborn from unrelated family referred to our clinic due to ambiguous genitalia and abdominal mass. Karyotyping of lymphocytes from peripheral blood was performed by conventional techniques. Genomic DNA from peripheral blood was extracted and the SRY region was amplified by polymerase chain reaction (PCR) using primers specific for the diagnosis of presence or absence of SRY gene.
Results: Karyotype analysis of three patients confirmed 46,XX karyotype without any numerical or structural chromosomal aberrations and peripheral blood DNA was negative for SRY gene.
Conclusion: Majority of the XX males carry SRY gene translocated to the X chromosome due to an illegitimate recombination between X and Y chromosomes. XX males without SRY gene have ambiguous to normal genitalia, show incomplete to complete masculinization and are infertile. The existence of SRY-negative males ruled out the prevailing notion that the mere presence of SRY determines maleness. Different hypotheses have been put forward to explain the occurrence of the SRY-negative XX males. Altered expression of genes crucial to gonadal development, such as *SOX9* and *SOX3*, may invert the expected embryonic plan. In conclusion, evidences from multiple studies suggest that SRY-negative XX maleness largely remains unexplained.
Key words: *Sex-reversal, SRY Gene, Infertility, Male.*

O-14
Detection of heterozygote mutation in *ALDH1A3* gene causing anophthalmia in a fetus

Dehghani MR[1], Dehghan Tezerjani M[2], Vahidi Mehrjardi MY[1], Hashemian Z[1], Metanat Z[3].
1. *Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Provincial Clinical Genetic Counseling Center, Zahedan University of Medical Sciences, Zahedan, Iran.*

Email: dehghani_dr@yahoo.com

Introduction: Anophthalmia is clinically characterized by absence of ocular tissue in one or both orbits. It may occur in isolation or as part of a syndrome that have complex etiology with chromosomal, monogenic and environmental causes. Studies indicate that the disease is heterogeneous and can be originated from mutations in different genes.
Materials and Methods: In this study, we performed genome wide single nucleotide polymorphism (SNP)-array analysis followed by homozygosity mapping and candidate gene sequencing in two families with three patients suffering from severe bilateral anophtalmia.
Results: We identified a homozygous missense mutation, causing a substitution of glycine (Gly) to arginine (Arg) at residue 237 of Aldehyde Dehydrogenase 1 (*ALDH1A3*) in the patients. The carrier mother from family 1 was pregnant and referred for PND. CVS was done before 12 weeks and DNA was obtained from chorionic villus using standard procedures. We detected same mutation in *ALDH1A3*.
Conclusion: Our report highlights the fact that subjects with mutations in *ALDH1A3* gene can also show eye anomalies, which has important implications for genetic counseling as well as the prenatal diagnosis of the disease. The variation might be suggestive of the presence of a founder effect in this area and population.
Key words: *Anophthalmia, Microphthalmia, ALDH1A3, Founder mutation, Consanguinity.*

O-15
Improving fluorescence in-situ hybridization (FISH)-based preimplantation genetic diagnosis/ screening (PGD/PGS)

Montazeri F[1, 2], Foroughmand AM[1], Kalantar SM[3], Aflatoonian A[3], Khalili MA[3], Fesahat F[3], Soleimani M[3, 4], Taheri F[3].
1. *Genetic Department, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*

Email: marjan.montazeri@gmail.com

Introduction: The goal of pre-implantation genetic diagnosis (PGD) is selecting and transfering embryos with no abnormalities in chromosomal number and structure, to increase the successful rate of in vitro fertilization (IVF). FISH-based PGD has become a very

controversial technique however it is an accepted and routine method in most IVF centers.

Materials and Methods: 180 biopsied blastomers from arrested embryos were assigned to three groups: group I (n=60), for analyzing one or two blastomeres for PGD using FISH; group II (n=60), for investigating the efficacy of three fixation methods, and group III (n=60), for studying the feasibility of carry out repeated FISH procedure in the same blastomer.

Results: Considering our results, it seems that analyzable embryos was significantly higher in two cell biopsy method comparing with one cell. Result from repeated FISH procedure showed after first round; 57 of 60, after second round; 52 of 60 and after third round; just 32 of 60 blastomers were analyzable. Blastomere fixation using first method showed better result compare to two other methods.

Conclusion: Our experience showed that improving FISH-based PGD procedure convert it to an efficient technique for detecting abnormalities in chromosomal number and structure and therefore, result in decreasing IVF failure in infertile patients.

Key words: *FISH, (PGD/PGS), Fixation method, Diagnostic accuracy.*

O-16
Investigating of *TSLP* C-847T polymorphism in patients with endometriosis

Poursadoughian Yaran A[1], Naji T[1], Shahhoseini M[2], Afsharian P[2].

1. *Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: a.poursadoughian@gmail.com

Introduction: Endometriosis is a chronic inflammatory disease, characterized by implantation and growth of endometrial tissue outside the uterine cavity. Multiple theories exist regarding its etiology and pathogenesis of disease is not clearly known. Indeed, dysregulation of the immune response toward endometriotic lesions has been noted in patients, including increased inflammatory cytokines and over reactive macrophages and neutrophils in the peritoneal cavity. One of these cytokine is Thymic Stromal Lymphopoietin (TSLP) that is a member of the 4-helix bundle cytokine family and a distant paralog of IL-7. Twenty-three polymorphisms have been reported for TSLP and one if its functional can be resulted froma promoter SNP (rs3806933), appear to contribute to Th2-polarized immunity through higher TSLP production. The purposeof this study is finding the impact of TSLP C-847T polymorphism in endometriosis patients.

Materials and Methods: A case-control study was designed. One-hundred patients with endometriosis and 100 Fertile women without any signs of endometriosis as a control group were enrolled in this study. TSLP promoter SNP (rs3806933) was genotyped using the polymerase chain reactions (PCR) followed by direct Sanger sequencing. Chi square method and SHEsis software were applied to statistical analysis of our results.

Results: The mean of age; BMI were 30.60±4.85 and 27.11±5.19 years; 24.87±3.24 and 27.23±4.40 kg/m^2 in endometriosis patients and control group respectively. CC, CT and TT genotypes were observed 9%, 61% and 30% in patients respectively, whereas they were 16%, 44% and 40% in control group respectively (p=0.046).

Conclusion: Endometriosis is a type of chronic inflammatory disease and this SNP (rs3806933) is very common in inflammatory disorders. According to our results, this polymorphism was significantly correlated with susceptibility of endometriosis in our studied population.

Key words: *Endometriosis, Polymorphism, Thymic Stromal Lymphopoietin, TSLP.*

O-17
Specific overexpression of NDRG2 tumor suppressor gene and investigation of its effects on proliferation, invasion and metastasis in LNCaP cell line and evaluation of its synergistic effect with radiotherapy and chemotherapy

Alizadeh Zarei M, Takhshid MA.

Department of Medical Biotechnology, Diagnostic Laboratory, Sciences and Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Email: mraziyehalizadeh91@yahoo.com

Introduction: NDRG2 has been recently identified as a promising candidate tumor suppressor in several human malignancies including prostate cancer (PCa). However, the specific overexpression of NDRG2 in hormone-dependent LNCaP cell line have not yet been reported.

Materials and Methods: Specific overexpression of NDRG2 was established by constructing a shuttle adenovirus containing specific promoter/enhancer. Cell viability was measured using MTT and colony formation assay and apoptosis was analyzed through flow cytometry. Migration and invasion was assessed using transwell chamber assay. MMP2 and MMP9 expression level was measured by real-time PCR. In this study, we also explored the synergistic effects of NDRG2 overexpression combined with X-radiation and docetoxel in LNCaP cell line.

Results: Specific overexpression of NDRG2 significantly inhibited LNCaP cell proliferation, induced LNCaP cell apoptosis, and decreased migration and invasion cells. Exogenous NDRG2 gene expression also downregulated the expression levels MMP2 and MMP9. NDRG2 overexpression synergizes radiotherapy and chemotherapy antitumor effects in LNCaP cells.

Conclusion: Our results indicate that NDRG2 overexpression could be a potential combined treatment strategy for prostate cancer.This findings may open up

avenues for further investigations to explore the future therapeutic use of NDRG2 in prostate cancer management.

Key words: *NDRG2, Prostate cancer, Gene therapy, Combination therapy, Synergistic effect.*

O-18
Promoter assay of the aromatase in human granulosa cells by luciferase assay

Hashemian Z[1, 2], Amiri-Yekta A[2], Hosseinkhani S[3], Shahhoseini M[2], Afsharian P[2].

1. *Department of Molecular and Cellular Biology, University of Science and Culture, Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Biochemistry, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: zhsh.1369@gmail.com

Introduction: Aromatase is the key enzyme of estrogen biosynthesis that encoded by *CYP19A1* gene. Aromatase gene has a wide regulatory area which contains 11 tissue-specific promoters expressed in different tissues such as granulosa cells. So far the transcription of aromatase gene in granulosa cells has not been clear, yet. One application of the luciferase assay is detecting the promoter activity, that luciferase gene is affected by its targeted promoter.

Materials and Methods: We amplified, purified and cloned four segments of targeted promoters and then each of them was inserted in pGL4.26 vector upstream of luciferase gene. After confirming the results by clony PCR, Enzymatic double digestion and Sanger-sequencing, the vectors were transfected into the primary cultured granulose cells (with or without of FSH) extracted from follicular fluid of women with normal foliculogenesis undergone ART. 48 hr later, the activity of luciferase was measured and compared between each group.

Results: The results shown just PII and PII/I.3 segments in the presence of FSH had significantly higher activity compare to others. The mean for pGl4.26-PII/I.3 +FSH and pGL4.26-PII +FSH groups only showed the significant difference with mean of pGL4.26 group as a control.

Conclusion: The present study showed that the activity of promoter PII in presence of FSH leads to express aromatase enzyme in human granulosa cells.

Key words: *Aromatase, Promoter, Granulosa cells, Luciferase assays.*

O-19
Expression patterns of *TIMP2* gene in preeclamptic women using cell free fetal RNA

Seydabadi S[1, 2, 3], Ramazanali F[3], Shahhoseini M[2], Favaedi R[2], Ghaheri A[4], Nikukar H[1, 5], Zamanian MR[2].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR,Tehran, Iran.*
3. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
5. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: sara.seydabadi68@gmail.com

Introduction: Preeclampsia is a pregnancy disorder with 5-10% prevalence and usually occurs in second or third trimester. Although the specific ethiology is unclear but there is substantial evidence for a pathogenic model of preeclampsia, where insufficient trophoblast invasion leads to incomplete remodeling of spiral arteries so perfusion of feto-placental unit decreases. The balance between *MMP/TIMP* genes is important in degradation and remodeling of extracellular matrix. Therefor their expression could be used as marker for PE diagnosis. Currently, the presence of cell free fetal RNA (cffRNA) in maternal plasma has been demonstrated, as a result we assumed that pregnancies complicated by preeclampsia will be associated with an abnormal expression of *TIMP2* gene cffRNA in the maternal plasma.

Materials and Methods: In this study whole blood have been collected from 20 preeclamptic women as a case group and 20 normal pregnant women as match controls in 28-32 wks of gestation age. Plasma was separated and cffRNA was extracted. Quantitative expression of *TIMP2* gene was evaluated by Real-Time PCR and then analyzed statistically.

Results: Results indicated that cffRNA expression of *TIMP2* gene in preeclamptic women was significantly increased compared to normal groups ($p \leq 0.05$).

Conclusion: To conclude increased cffRNA expression of *TIMP2* gene in plasma of preeclamptic women may be associated with pathogenesis of disease. More work is needed.

Key words: *Preeclampsia, Cell free fetal RNA, Expression, TIMP2.*

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O-20
Association of *IL-17A* and *IL-17F* gene polymorphisms with recurrent pregnancy loss in Iranian women

Najafi S[1], Hadinedoushan H[2], Eslami G[3], Aflatoonian A[4].

1. *Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*

2. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Parasitology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Research and Clinical Center of Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: soheil.najafi@gmail.com

Introduction: Recurrent pregnancy loss (RPL) is defined as the occurrence of two or more miscarriages before the 20th week of pregnancy. T helper17 cells are a novel subset of T cells, which secrete IL (Interleukin)-17 and are known to be involved in inflammation, autoimmunity and rejection of nonself tissues.
Materials and Methods: A case-controlled study was performed on two groups consisting of 85 healthy women with at least one delivery and 85 women with the history of two or more RPLs. The frequency of IL-17A rs2275913 and IL-17F rs763780 polymorphisms were determined by PCR-RFLP.
Results: In the RPL group, the genotypes frequencies of rs2275913 polymorphism were GG (8.2%), AG (30.6%), and AA (61.2%) and in the control group, were GG (3.5%), AG (42.4%) and AA (54.1%). Statistical analysis showed no significant difference between the genotypes of AA, AG and GG in the two groups (p=0.1). The genotypes frequencies of rs763780 polymorphism were TT (43.5%), TC (49.4%) and CC (7.1 %) in the RPL group; whereas the frequencies were TT (25.9%), TC (70.6%) and CC (3.5%) in the control group. Statistical analysis revealed a significant difference in the TT, TC, and CC genotypes frequencies between the case and the control groups (p=0.01).
Conclusion: Our findings indicate that IL-17F polymorphism, rs763780, might be associated with a high risk of RPL in Iranian women.
Key words: *IL-17, Genotyping, Polymorphism, Recurrent pregnancy loss.*

O-21
MSC administration induces a privileged tolerant microenvironment at the fetal maternal interface in the abortion prone mouse model

Salek Farrokhi A[1], Zarnani AH[2], Moazzeni SM[1].
1. *Department of Immunology, Tarbiat Modares University, Tehran, Iran.*
2. *Nanobiotechnology Research Center, Avicenna Research Institute, Tehran, Iran.*

Email: A.salek@modares.ac.ir

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy. The mechanisms underlying immune tolerance during pregnancy are poorly understood. In this regard, Treg seem to play an important role in mediating maternal tolerance to the fetus. MSCs have been shown to modulate immune responses by the de novo induction and expansion of Treg cells.
Materials and Methods: The MSCs were derived from the abdominal fat of CBA/J mice. On the day 4.5 of gestation MSCs was administered (i.p) to mice in the test group. On day 13.5 of the gestation the percentage of CD4+CD25+ FoxP3+ cells analyzed by flow cytometry in the spleen and lymph node. The mRNA level of Foxp3, HO-1, PD-1, IL-10 and TGF-β genes in the decidua and placenta were determined by Real-Time PCR.
Results: The MSC group presented significantly diminished abortion rates as compared to abortion group, as expected (5% vs. 29.83%, p=0.0045). Our result showed that MSCs treatment augmented levels of CD4+CD25+foxp3+ cells in the lymph node (p=0.0001) and remarkably up-regulated the expression of Foxp3, HO-1, PD-1, IL-10 and TGF-β genes in the decidua and placenta.
Conclusion: Here, we show for the first time that high levels of CD4+CD25+Foxp3+ cells induced by MSCs administration reduced abortion rate in the abortion prone mouse model. Our data suggest that MSCs treatment is able to create a privileged tolerant microenvironment at the fetal maternal interface.
Key words: *Recurrent spontaneous abortion, Mesenchymal stem cell, Regulatory T cell, Immune tolerance.*

O-22
The effect of mesenchymal stem cells therapy on uterine natural killer cells phenotype and cytokine production in abortion-prone mouse model

Rezaei F, Moazzeni SM, Mohseni M.
Department of Immunology, Tarbiat Modares Universty, Tehran, Iran.
Email: diba.rezaei@yahoo.com

Introduction: Uterine natural killer cells (uNK) are the major population of immune cells in the maternal - fetal interface and play an important role in establishment and maintenance of normal pregnancy. Recurrent spontaneous abortion is one of the most common complications of pregnancy which in many cases is related to the immune system disorders. We have shown that mesenchymal stem cells (MSCs) therapy could reduce the abortion rate in abortion prone mice. In this study we aim to evaluate the effect of MSCs therapy on uNK cells phenotype and their cytokine profile.
Materials and Methods: MSCs were injected (IP) at day 4 of gestation to female CBA/J mice following their mating with DBA/2 male. In control group PBS was injected and CBA/J x BALB/c mating was also used as normal pregnancy. On day 12.5 of pregnancy embryo resorption rate was determined and decidual cells were isolated by enzymatic digestion. The immunophenotype and intracellular cytokine production by NK cells were examined through flow cytometric analysis.
Results: MSCs administration dramatically decreased embryo resorption rate compared with control groups. Also MSCs could affect the phenotype of NK cells in uterine and changed the pattern of activating and inhibitory receptor on cell surface to more regulatory

types. The cytokine profiles of NK cells will also changed in accordance with their phenotype.

Conclusion: These findings indicate that administration of MSCs improved pregnancy outcome and correct the functions and phonotype of uNK cells in abortion prone mice. However, the changes in other properties of uNK cells and other aspects of immune systems are remained to be determined and is under investigation in our laboratory.

Key words: *Recurrent spontaneous abortion, Mesenchymal stem cell, NK cells, Cell therapy.*

O-23

The study of T helper cell subsets and the related cytokines in infertile women undergoing IVF before and after seminal plasma exposure

Kanannejad Z[1], Keshtgar S[2], Namavar Jahromi B[3], Azada M[1], Gharesi-Fard B[1], Zare M[1].

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran.*
2. *Department of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran.*

Email: zkanannejad@gmail.com

Introduction: Infertility is a multi-factorial disorder and immunological factors, including T cells and their related cytokines, might involve in predisposing a couple to infertility. In vitro fertilization (IVF) is a well-known method for treatment of infertility.

Materials and Methods: This study was performed on 19 couples with unexplained infertility undergoing IVF treatment. Among the studied group, 9 and 10 couples had successful and unsuccessful IVF outcomes, respectively. This study was carried out by Real Time PCR (RT-PCR) technique.

Results: The results indicated that before seminal plasma exposure, expressions of T-bet (p=0.007), IFN-γ (p=0.013), and TNF-α (p=0.017) were increased, while those of GATA3 (p≤0.0001), Foxp3 (p=0.001), and IL-35 (p≤0.003) were decreased in the infertile women with IVF failure compared to those with successful IVF outcomes. After seminal exposure, expressions of T-bet (p=0.02), Rorc (p=0.0001), TNF-α (p=0.001), Foxp3 (p=0.02), and IFN-γ (p=0.001) were increased in the unsuccessful IVF group, while expressions of Foxp3 (p=0.02), Rorc (p≤0.0001), IL-23 (p=0.04), IL-17 (p=0.02), IL-6 (p≤0.0001), TGF-β (p=0.01), and IL-35 (p≤0.0001) were increased in the successful IVF group.

Conclusion: In summary, the results indicated that IVF failure was associated with imbalance in Th1/ Th2/ Th17/ Treg responses. Moreover, the results showed that seminal plasma might have a positive effect on the IVF outcome via deviation of peripheral blood T cells subsets.

Key words: *Infertility, T helper cells, Transcription factor, Cytokine, IVF.*

O-24

Innate immune responses in granulosa cells of infertile endometriosis women

Taghavi SA[1], Gholamnezhadjafari R[2], Amirchaghmaghi E[3], Salman Yazdi R[3], Karimian L[3], Ashrafi M[3], Aflatoonian R[3].

1. *Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandarabbas, Iran.*
2. *School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
3. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, ACECR, Royan Institute, Tehran, Iran.*

Email: vahab.taghavi@gmail.com

Introduction: Endometriosis is a common gynecological condition that is described by the presence of endometrial tissue fragments outside of the uterine cavity. Endometriosis is associated with increased number of leukocytes and increased concentrations of interleukins (IL) -6, IL-1β, IL-10, TNF- α and nuclear factor kappa-B (NF-kB) that all of them are known downstream targets of toll-like receptors (TLRs).

Materials and Methods: Twenty infertile endometriosis patients and 20 normal women underwent controlled ovarian stimulation. Follicular fluid (FF) was collected from patients and a series of isolation and purification techniques was performed, involving Ficol density gradient centrifugation. Cellular pellet was used for evaluation of TLRs and their signaling pathway genes expression by Q- PCR. Follicular fluid was used for determination of cytokines protein expression by ELISA.

Results: *TLR1, 5, 6, 7, 8, 10, MYD88, NF-κB, IL-10* and *TGF-β* genes expression were significantly higher in endometriosis compare to control (p≤0.05). *TLR3, 9, INF-β* genes expression were significantly lower in endometriosis than control (p≤0.05). The expression of *TLR2, 4, TIRAP, TRIF, TRAM,* and *IRF3* genes revealed not significant difference in both groups. IL-6, IL-8 and MIF protein expression were significantly higher in FF of endometriosis than normal women (p≤0.05).

Conclusion: Our data would be recommended the involvement of TLRs in pathogenesis of endometriosis. In addition, alteration of TLRs expression in the granulosa cells of endometriosis patient is responsible for poor oocyte quality and diminished fertilization rate through changes in the FF cytokine profile.

Key words: *Endometriosis, Follicular cells, Infertility, Innate immunity, TLR.*

O-25

The immunomodulatory effects of decidual cell from resorbtion and non-resorbtion decidua on dendritic cell function

Eskandarian M, Jahangiri AH, Hyaftiyan A, Moazzeni SM.

Department of Iimmunology, Tarbiat Modares University, Tehran, Iran.

Email: maryamskandaryan@yahoo.com

Introduction: Dendritic cells (DCs) can acquire immunogenic or tolerogenic properties depending on tissue environmental factors and cell- cell contact in feto-maternal interface. We aimed to determine the immunomodulatory effects of decidual cell from resorbtion and non-resorbtion decidua in prone abortion mice on DC functions.
Materials and Methods: DCs were differentiated from mouse bone marrow (BM) cells in the presence of DC differentiation cytokines, GM-CSF and IL-4. The decidual cells were cultured from abortion and non-abortion decidua. DCs was added to selected cultures of decidua cells. DC immunophenotype was evaluated by the expression of MHCII, CD40 and CD86. Dextran uptake was also studied for the assessment of phagocytotic ability of the generated DCs.
Results: Our results indicated that treatment of dendritic cells with decidua cell from resorbtion decidua significantly increased MHCII, CD40 and CD86 expression by BMDCs. Diminished endocytic capacity was also observed in BMDCs that were treated with resorbtion decidua.
Conclusion: It can be concluded that cell- cell contact and decidua-secreted factors, by altering DC functions, can determine the pattern of immune responses at the feto-maternal interface and, subsequently, pregnancy outcome.
Key words: *Dendritic cells, Decidua, Resorbtion.*

O-26
Uterine natural killer cell and human leukocyte antigen-G1 and human leukocyte antigen-G5 expression in vaginal discharge of threatened-abortion women

Sadatshobeiri S, Abediankenari S.
Department of Immunology, Mazandaran University of Medical Sciences, Sari, Iran.
Email: saeideshobeiri@gmail.com

Introduction: The immunotolerant human leukocyte antigen-G (HLA-G) molecules have a major role in fetal-maternal tolerance during pregnancy. Interaction between these molecules and uterine natural killer (uNK) cells inhibitory receptors prevents NK cell invasion against fetus trophoblast cells.
Materials and Methods: In a case-control study, we investigated 30 threatened-abortion women with bleeding or spotting less than 20 wk of pregnancy as compared to 30 normal pregnant women. uNK cells percentage was assessed by flow cytometry. Furthermore, we evaluated HLA-G1 and HLA-G5 isoforms expression by Real-Time PCR in these groups.
Results: The results of this study showed that threatened-abortion women had increased uNK cells and decreased T cells percentage in vaginal discharge in comparison with normal pregnant women.
Conclusion: The increase of uNK cells level with the decrease of HLA-G expression in vaginal discharge of threatened-abortion pregnant women is an indicator of mother's immune dysregulation. It is concluded that HLA-G expression level with uNK cells percentage can be determined as a diagnostic marker for threatened-abortion women.
Key words: *uNK, HLA-G, Vaginal discharge, Threatened-abortion.*

O-27
Investigating the association of IL-27 with susceptibility to preeclampsia in Iranian women

Aramesh A[1], Namavar Jahromi B[2], Batebi A[2], Samadi M[1], Gharesi-Fard B[1].
1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Gencology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: azamaramesh@gmail.com

Introduction: Preeclampsia (PE) is one of the most serious and important disorder of the human pregnancy with high rate of mortality and morbidity for the fetus and mother. Several etiological factors including immunological and genetic factors are involved in the onset of the disease. Up regulation of IL-27 has been reported in placental tissue from pre-eclamptic women compared to normal pregnant women but the role of IL-27 is not investigated in PE.
Materials and Methods: This case-control study was done on 199 PE patient and 228 age and gestational matched healthy women as control group. IL-27 rs153109 and rs17855750 SNPs were genotyped using PCR-RFLP method. Moreover the level of IL-27 were determined in 40 PE and 45 healthy women using ELISA method.
Results: Statistical analysis indicated that there were no differences in genotype, allele and genotype combination frequencies regarding the studied SNPs between cases and controls. The plasma level of IL-27 was elevated in patients and in mild form of the disease compared with controls (p=0.009 and p=0.006, respectively).
Conclusion: It seems that IL-27 rs153109 and rs17855750 SNPs are not different in PE and healthy women.
Key words: *Preeclampsia, IL-27, PCR-RFLP.*

O-28
Evaluating anti-tumor activity objects in peripheral blood lymphocytes of women with polycystic ovary syndrome (PCOS) by co-culture with SKOV3, A2780 (ovarian tumor cell lines)

Hajiaghayi M.
Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: mehri.hajiaghayi@gmail.com

Introduction: Polycystic ovarian syndrome (PCOS) is a proinflammatory state that underpins the development

of metabolic aberration and ovarian dysfunction in the disorder. Chronic inflammation and increased levels of androgens in this group of patients and their impact on the immune system, may be able to disrupt the antitumor activity and thus increase the risk of developing malignancies including ovarian cancer.

Materials and Methods: Peripheral blood mononuclear cells of 50 patients with PCOS and healthy samples were purified by Ficoll density gradient centrifugation. We then measured cell proliferation and concentrations of cytokines TNF-α at different time intervals (48 and 72 hr) after co-culture of ovarian (SKOV3, A2780) and breast (MCF-7, MDA-468) tumor cell lines with PBMC in indirect contact of transwell system.

Results: Proliferative response of executive cells during stimulation with tumor cell lines despite lower average in the control group, was not statistically significant between patients and healthy subjects. After 72 hr the proliferation was significantly higher than after 48 hr ($p \leq 0.01$). The production of TNF-α in co-culture of A2780 cell lines significantly increased in the patient group in time compared to the controls ($p \leq 0.05$).

Conclusion: Low levels of chronic inflammation in patients with PCOS confirmed increased proliferative response of effector cells and TNF-α levels compared to healthy individuals. However, an increased risk of cancers in patients with PCOS requires investigation of other aspects of anti-tumor responses in vitro, with higher sample volume.

Key words: *Polycystic ovarian syndrome, Chronic inflammation, Ovarian tumor cell lines, SKOV3, A2780, Co-culture.*

O-29
Immunologic dysregulation in polycystic ovary syndrome

Tavakoli M.
Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
Email: tavakolim77@gmail.com

Introduction: Polycystic ovary syndrome (PCOS) is one of the common endocrine disorders with heterogonous etiology in women of reproductive ages that could be associated with reproduction complications such as infertility and recurrent abortion as well as insulin resistance, negative effects on glucose metabolism and cardiovascular diseases.

Materials and Methods: Here is a brief review of immunologic findings in PCOS patients providing by searching in pubmed.

Results: Regarding the role of estrogen in autoimmune diseases it seems that hyperandrogenism in these patients could have a protective role against autoimmunity but low levels of progesterone may lead to organ and non-organ specific autoantibodies production. Hormonal alterations and metabolic disorders might be related to chronic inflammation. TNF-α and IL-6 polymorphisms, increased level of IL-18, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin, decreased level of osteoprotegrin, reduced nitric oxide production are noticed as probable etiologies of insulin resistance, hyperandrogenemia and increased systemic vascular resistance in these patients.

Conclusion: Finding more about the immunopathophsiology of this long-life disease could be useful in finding more effective treatments.

Key words: *Polycystic ovary syndrome, Infertility, Recurrent abortion, Immunology.*

O-30
Adhesion molecules, implantation and infertility

Ghafourian M[1], Nikbakht R[2], Ghalambor Dezfuly F[2], Chinipardaz R[3].
1. *Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
2. *Department of Obstetrics Gynecology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
3. *Department of Statistics, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*

Email: ghafourianbm@gmail.com

Introduction: Adhesion molecules mediated cell-cell and cell-matrix interactions regulate different type of cellular activities. Recent attention has focused on the expression of some adhesion molecules within endometrial tissue as a marker of uterine receptivity during the implantation window.

Materials and Methods: In a case-control study 30 endometrial biopsies from hysterectomies with nonendometrial pathology and 30 endometrial samples by uterine curetting from infertile women in secretary phase at implantation time were collected. The samples were stained with six monoclonal antibodies against β1 integrin (VLA-1 to VLA6) and β3 integrin subunit by immunohistochemical technique and then assessed semiquantitively by microscope on different compartments including glandular epithelial cells, vessels, lymphocytes, macrophages and stromal cells. Chi- Square test was used to compare the expression and defect of beta1 and beta3 integrin molecules between two groups in different compartments.

Results: The majority of glandular epithelial cells and stromal cells expressed VLA-1 and VLA-4 integrin molecules in fertile endometrium. However, the reactivity with them reduced significantly in both glandular epithelial cells and stromal cells in infertile women ($p \leq 0.5$).

Conclusion: VLA1, VLA-4 and beta3 integrin molecules may contribute in uterine endometrial receptivity at the time of the implantation window. A therapeutic potential approach in improving endometrium receptivity of infertile women by up-regulation of some integrins suggested.

Key words: *Integrins, Endometrium, Implantation, Infertility.*

O-31

MMP9 promoter polymorphism (-1562 C/T) does not affect the serum levels of soluble MICB and MICA in breast cancer

Bargostavan MH[1], Eslami G[2], Esfandiari N[3], Shams Shahemabadi A[4].

1. *Department of Immunology, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Parasitology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Imam Ali Clinic, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: alishams@ssu.ac.ir

Introduction: The role of Matrix Metalloproteinase 9 (MMP9) in tumor invasion and progression is prominent. A single nucleotide polymorphism (SNP) in the promoter region of *MMP9* (-1562 C/T) increases the transcription and expression of this gene. On the other hand, MHC class I chain-related protein A and B (MICA/B) in soluble forms may impair tumor immunogenicity by reducing Natural Killer Group 2D (NKG2D) densities on NK cells and MMP9 enzyme activity has a prominent role in shedding of MICA/B. The association between *MMP9* (-1562 C/T) polymorphism and serum MICA/B level in breast cancer patients was investigated in this study.

Materials and Methods: In this case-control study, 105 patients with breast cancer and 100 healthy age-matched women were selected from Yazd hospitals, Iran. The polymorphism of *MMP9* (-1562 C/T) was determined by PCR-RFLP. Concentration of MICB and MICA in the sera of breast cancer patients and healthy women were measured using ELISA method.

Results: The frequency of CC, CT and TT genotypes and T allele of the *MMP9* (-1562 C/T) did not show significant differences between breast cancer patients and healthy controls ($p>0.05$). On the other hand, the mean serum levels of MICB and MICA were significantly elevated in patients compared with healthy individuals ($p<0.05$). In patients with *MMP9* CC genotype, the mean serum MICB concentration was significantly higher than those patients with CT polymorphism ($p<0.05$). Although the mean of blood MICA concentration in patients with the CT genotype was higher than those patients with CC genotype, the difference was not statistically significant.

Conclusion: The T allele of the *MMP9* (-1562 C/T) does not show a correlation with serum levels of MICA and MICB in breast cancer patients.

Key words: *Breast Cancer, Matrix Metalloproteinase 9, MHC Class I-Related Chain A.*

O-32

Can regulatory T cells use as a novel target in therapeutic abortion?

Badiee M, Ghafourian M, Ghadiri A, Nikbakht R, Chinipardaz R.

Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Email: Badiee.minayi@gmail.com

Introduction: Pregnancy is a complex event that the maternal immune system tolerates the foreign antigens of the fetus, and immune tolerance occurs. Regulatory T cells modulate the function of immune system to retain homeostasis. Inadequate immunoregulatory mechanisms during pregnancy or disruption in this immune tolerance may lead to recurrent pregnancy loss (RPL), and usually occurs in the first trimester of pregnancy.

Materials and Methods: Flow cytometric assay using monoclonal antibodies was performed to identify CD4+ CD25+ regulatory T cells (CD25dim and CD25 bright), FoxP3 expression was evaluated using real-time PCR method, and anti-inflammatory cytokines including IL-10 and TGF-β were determined using ELISA kits. The independent-samples T test was applied for statistical analysis.

Results: The percentage of CD4+ CD25 bright T cells was significantly lower in women with RPL ($p<0.5$).

Conclusion: These observations demonstrate that the decrease of regulatory agents including CD4+CD25 bright T cells, FoxP3 expression, and TGF-β level may disturb immune tolerance and homeostasis during pregnancy and induce abortion in RPL women. Due to the protective role of regulatory T cells in pregnancy status, it suggests that these cells may be a novel target for treatment of women suffering RPL problem.

Key words: *Recurrent pregnancy loss, CD4+CD25+ T cells, FoxP3, TGF-β, IL-10, Flow cytometry, Real-time PCR, ELISA.*

O-33

Evaluation of some common risk factors and *HLA-G* 14 bp gene polymorphism in relation to sHLA-G levels in preeclamptic and normal pregnancies

Rokhafrooz S[1], Ghafourian M[2], Ghadiri A[3], Hossaini SH[1], Najafian M[4], Daraei N[1].

1. *Department of Immunology, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*
2. *Department of Immunology, Fertility Infertility and Perinatology Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*
3. *Department of Immunology, Faculty Medicine, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*
4. *Department of Obstetrics Gynecology, Fertility Infertility and Perinatology Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*

Email: rokhafrooz77@gmail.com

Introduction: Preeclampsia (PE) affects 3-10% of pregnancies that is a major cause of fetal-maternal morbidity and mortality. Human leukocyte antigen-G (HLA-G) is a class Ib molecule expressed on the extra-villous trophoblast and seems to have immunomodulatory functions during pregnancy.

Materials and Methods: A number of 150 healthy pregnant women and 150 patients with PE had been

genotyped for the 14 bp insertion/deletion polymorphism in exon 8 of *HLA-G* gene, and the serum levels of sHLA-G protein were measured using the enzyme-linked immunosorbent assay. Also, the two groups compared in terms of maternal age, BMI and hemoglobin, gestational age, PE season and child weight at birth.

Results: The maternal age, gestational age, maternal hemoglobin and maternal BMI were significantly associated with risk of PE ($p \leq 0.0001$), while the PE season did not reach the statistically significant in this regard. Data showed that the PE syndrome was not associated with *HLA-G* 14 bp genotype. But, the serum levels of sHLA-G in PE patients were significantly lower than in healthy pregnant women in the third trimester. While, no significant association was observed between the 14 bp genotype and serum sHLA-G level.

Conclusion: The data support a role for sHLA-G level in maternal blood serum and suggest that maternal age, maternal hemoglobin and maternal BM may contribute to impaired human extra-villous trophoblast invasion and pathogenesis of preeclampsia. These findings provide new insights into the diagnosis and treatment of PE.

Key words: *Preeclampsia, Risk factors, Polymorphism, sHLA-G.*

O-34

Evaluation of Th1 and Th17 cells cytokines in women with a history of recurrent spontaneous abortion

Varghaiyan Y[1], Hadinedoushan H[1], Aflatoonian A[2].

1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: yase.V@yahoo.com

Introduction: Various immunological abnormalities have been reported in women with recurrent spontaneous abortion (RSA) of unknown aetiologies including autoimmune abnormalities and increased cellular immunity. T helper (Th) 7 and Th1 cells play a central role during inflammation. Th1 cells product are mainly cytokines interferon gamma (IFN-γ) and interleukin (IL)-2 and Th17 cells products are mainly cytokines IL-17A, F and IL-22.

Materials and Methods: This study was carried out as a case control study on three different groups. Group I consisted of 30 normal fertile healthy women with at least one delivery. Group II consisted of 30 women with a history of two or more RSA with at least two months after the last abortion. Group III consisted of 30 women with a history of two or more RIF with at least two months after last failed in vitro fertilization cycles. We determined the levels of IL-17A,F and IFNγ in serum and peripheral blood mononuclear cells stimulated with the phytohemagglutinin by ELISA method.

Results: There was no significant difference in 3 groups regarding age of women (30.47±4.7 [control], 29.27±5.3 [RSA], and 32.5±5.8 yr [RIF]). The mean of abortion was 3.1±0.24 (range 2-8) and RIF was 3.2±0.26 (range 2-8). IL-17A,F level in cell culture supernatant of PBMCs was significantly higher in RSA group (84.7±21.3 pg/ml) as compared with those of controls (28.4±8 pg/ml) ($p=0.01$). IL17 A,F concentration showed positive correlation with IFNγ ($r=0.455$, $p=0.015$). IFNγ level in cell culture supernatant of PBMCs was significantly higher in RSA women (186.5±30.4 pg/ml) as compared with those of controls (88.06±21.4 pg/ml) ($p=0.005$), also was significantly higher in RIF group (162.8±31.4 pg/ml) as compared with those of control ($p=0.03$).

Conclusion: Our findings showed that Th17 and Th1 cytokines increased in women with a history of RSA and RIF as compared to normal women. These cytokines may be considered as a risk factor for RSA in Iranian women.

Key words: *Recurrent spontaneous abortion, IL-17, IFN-gamma, Repeated implantation failure.*

O-35

Association of 14-bp insertion/deletion polymorphism of *HLA-G* gene with idiopathic recurrent miscarriages in Yazd, Iran

Arjmand F[1], Samadi M[2].

1. *Department of Molecular Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.*
2. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fateme.arjmand@yahoo.com

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 and/or deletion polymorphism in exon-8 has a possible role in HLA-G expression.

Materials and Methods: In this study, genomic DNA from 200 RM patients and 200 normal fertile control individuals using the routine salting out method were isolated. Exon-8 of *HLA-G* gene of the two groups were amplified using polymerase chain reaction and analyzed by electrophoresis on 10% non-denaturing poly-acrylamide gel electrophoresis containing ethidium bromide and visualized under ultraviolet light. *HLA-G* allele frequencies and genotypes in RM women and the fertile control group were compared using a Chi-square test.

Results: The results showed that there was a difference in allelic frequencies of 14-bp insertion polymorphism between fertile controls and RM patients; the frequency of +14 bp/14 bp heterozygotes was significantly higher in RM patients as compared with fertile controls. Furthermore, the frequency of +14-bp insertion allele was significantly higher in those with RM as compared with normal fertile controls.

Conclusion: From the findings here, it was concluded that a 14-bp insertion/deletion polymorphism in exon 8 could play a possible role in recurrent miscarriages.

These results might ultimately be of significance for clinicians and those involved in understanding infertility and RM.

Key words: *14-bp insertion/deletion polymorphism, HLA-G, Recurrent miscarriage, Recurrent spontaneous abortion.*

O-36

Evaluation of PD-1 inhibitory molecule on regulatory T cells in women suffering preeclampsia in comparison with normal pregnancy

Daraei N[1], Ghafourian M[2], Ghadiri A[3], Najafian M[4], Rokhafrooz S[5].

1. *Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
2. *Fertility Infertility and Perinatology Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*
3. *Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
4. *Fertility Infertility and Perinatology Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.*
5. *Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

Email: Nahiddaraei@yahoo.com

Introduction: During pregnancy, the maternal immune system is tolerant to foetal antigens via the engagement of immune regulatory mechanisms. Failure in regulating the maternal immunity to foetal antigens may lead to preeclampsia (PE). Exhausted Tregs express CD279 or programmed death receptor 1 (PD-1), a negative regulatory molecule that is associated with limited proliferative capacity and reduced immune suppression.

Materials and Methods: In this case-control study 37 women with PE on average the 34th gestational week of pregnancy and 40 women with normal pregnancy, age-matched at an average 34-36th gestational week were enrolled. Peripheral mononuclear cells from EDTA blood of both groups were separated by ficoll- Paque and stained with flurochrome-conjugated antibodies against human CD4, CD25 and CD279 markers and analyzed by three-color flow cytometry .

Results: The results showed the percentage of Tregs reduced in preeclampsia women compared with normal pregnant women, while the percentage of exhausted Tregs (Treg PD-1+) enhanced significantly in preeclampsia ones in relation to normal control group.

Conclusion: Increased PD-1 (CD279) molecule on Treg cells may involved in pathogenasis of preeclampsia, so the use of PD-1 as therapeutic target could be recommended in PE treatment.

Key words: *PD-1, Exhausted Treg, Flow cytometry, Preeclampsia.*

O-37

The expression of complement regulatory molecules in feto-maternal interface changes following MSCs therapy

Shahgaldi Sh, Moazzeni SM.

Department of Immunology, Tarbiat Modares University, Tehran, Iran.

Email: shahab.shahgaldi@modares.ac.ir

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy. The mechanisms underlying immune tolerance during pregnancy are poorly understood. In this regard, complement activation play an important role in the development of miscarriages. We showed that MSC therapy could reduce the abortion rate through modulation of immune responses.

Materials and Methods: Adipose tissue-derived mesenchymal stem cells (AT-MSCs) were isolated from the abdominal fat of CBA/J mice. On the 4.5th day of gestation, the test group received an IP injection of 1×10^6 of AT-MSCs. On the 13.5th day of gestation, tissue samples (placentae and deciduae of pregnant mice) were analyzed to measure the expression levels of Crry and Adipsin using real-time PCR.

Results: As expected, not only the resorption rate was significantly lower in the test group as compared to the control group, but also the average weight of fetuses in the test group was higher than that of fetuses in the control group. Moreover, the data obtained from real time PCR analysis demonstrated that the expression of Adipsin decreases while the expression of Crry increases in the placenta and decidua of abortion prone mice upon MSC administration.

Conclusion: Here, we show for the first time that adoptive transfer of MSCs contains fetal rejection and improves fetal developmental conditions in abortion-prone mice by modulating the expression of complement regulatory molecules Adipsin and Crry.

Key words: *Mesenchymal stem cell, Recurrent spontaneous abortion, Cell therapy, Crry, Adipsin.*

O-38

Sperm DNA damage can change the immunological crosstalk between sperm and female reproductive tract

Zandieh Z[1], Amjadi FS[1], Aflatoonian R[2].

1. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran. Iran.*
2. *Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: zandie.dvm@gmail.com

Introduction: Sperm DNA damage is a useful biomarker for male infertility which is associated with reduced embryo quality, fertility and pregnancy rate. TLRs are the major compartment of innate immune system. It is well established that microbial PAMPs are ligand for TLRs. However, it is becoming clearer that certain locally produced endogenous substances can also stimulate TLRs like reactive oxygen species (ROS). In addition, apoptosis and ROS are the most discussed causes of DNA damage.

Materials and Methods: Fresh semen samples were obtained from unexplained and recurrent implantation failure infertile couples with DNA fragmentation more than 30% and healthy donors with DNA fragmentation less than 5%. All these semen samples, after washing were co-incubated with human fallopian tube cell line. TLRs, their signalling pathways, as well as inflammatory cytokine production in human fallopian tube cells were evaluated by quantitative PCR, ELISA and TLR PCR array kit.

Results: Analysis of the results showed that the mean relative expression of TLRs were higher significantly in response to sperm with high DNA fragmentation than low ($p \leq 0.05$). Also, MYD88 dependent pathway had higher expression comparing with MYD 88-independent pathway. Besides, the vast majority of adaptors, effectors and member of NFkB, Jak/stat and cytokine mediated signalling pathway were intermediately to highly expressed in high DNA fragmented than low one.

Conclusion: Sperm DNA damage plays an important role in immunological interaction of sperm with female reproductive tract. Excessive ROS production causes lipid peroxidation and oxidative DNA damage, which leads to DNA fragmentation. Besides, fatty acids and reactive oxygen species (ROS) are the endogenous ligands of TLRs. Maybe, by this mechanism, DNA fragmentation can increase TLR expression and more production of inflammatory cytokines as well as causes infertility in high DFI infertile men. So, evaluation of DNA damage should be considered in treatment of these patients due to excessive Inflammatory cytokine production.

Key words: *TLR, Sperm, DNA fragmentation, Female reproductive tract.*

O-39
Association of TSLP and TSLP receptor expression levels with endometriosis

Habibi S[1], Amirchaghmaghi E[2], Ramazanali F[3], Aflatoonian R[3], Afsharian P[4], Shahhoseini M[4].

1. *Faculty of Basic Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
2. *Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR,Tehran, Iran.*
3. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Department of Genetics, Royan Institute for Reproductive Biomedicine, Reproductive Biomedicine Research Center, ACECR, Tehran, Iran.*

Email: habibi.soolmaz@yahoo.com

Introduction: Endometriosis as a chronic inflammatory disease characterized by implantation and growth of endometrial tissue outside the uterine cavity. Based on the immunological aspects of endometriosis, the T helper 2 (Th2) immune response which is under the control of different cytokines including thymic stromal lymphopoietin (TSLP) is activated and has been suggested to promote this chronic inflammatory disease. TSLP is an interleukin-7 (IL7) -like cytokine that triggers dendritic cell- mediated Th2 inflammatory responses. The receptor of this cytokine (TSLPR) which is also known as cytokine receptor-like factor 2 (CRLF2) is a heterodimeric cytokine receptor consisting of the IL-7 receptor alpha chain (IL-7Rα) and a TSLP-specific receptor chains. The present study aimed to elucidate the mRNA expression level of TSLP and TSLPR encoding genes in endometrial tissues of patients with endometriosis compared to women without endometriosis.

Materials and Methods: In this study, 15 patients with endometriosis and 16 normal women between 20-45 yr old were enrolled after diagnostic laparoscopy. Women with any other abnormalities were excluded. Informed consent was obtained from all women. Ectopic endometrial biopsies were obtained through laparoscopic procedure, eutopic and control biopsies were obtained by pipelle. The expression of TSLP, CRLF2, IL7Rα in normal, eutopic, and ectopic endometrial samples were evaluated quantitatively by real-time-PCR. Gene expression data were analyzed based on 2- ΔΔct to estimate the relative fold change values. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 21 software.

Results: Quantitative PCR analysis showed that the mRNA expression levels of TSLP and TSLPR (CRLF2, IL7Rα) were significantly increased in ectopic tissues of patients with endometriosis compared to eutopic tissues and control group.

Conclusion: These data collectively identify TSLP as a candidate gene critically involved in development of endometriosis beyond its role in promoting Th2 responses.

Key words: *Endometriosis, Immune response, Thymic Stromal Lymphopoietin (TSLP).*

O-40
Investigation of T-helper subsets balance in pre-eclampsia

Gharesi-Fard B, Mobasher-Nejad F, Nasri F, Hesampour F.

Department of Immunology, Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Email: gharesifb@sums.ac.ir

Introduction: Preeclampsia (PE) is known as a main factor in fetomaternal mortality, which might affect 2-8% of all pregnancies after the twentieth week of gestation. T helper cells are essential in maintaining normal pregnancy and developing PE. In the present study the levels of transcription factors and cytokine gene expression of Th1/Th2/Th17/Treg subsets within decidual and chorionic layers of placentas from 15 PE-afflicted and 15 healthy Iranian women in their third trimester of pregnancy.

Materials and Methods: Using quantitative real-time PCR(Q-PCR), the participants were compared regarding expression of T-bet, GATA-3, ROR-γt, Foxp3, and cytokines, such as IL-1, IL-6, TNF-α, IFN-γ, IL-4, IL-31, IL-17, IL-23, TGF-β1, TGF-β2, TGF-β3, and IL-35, at mRNA levels within placenta.

Results: According to the results, Foxp3 and GATA-3 were significantly down regulated, while T-bet was up regulated in PE deciduae compared to the control group ($p \leq 0.0001$, $p \leq 0.02$, and $p \leq 0.01$, respectively). Concerning the chorionic samples, Foxp3 significantly decreased, while ROR-γt increased in the PE placentas compared to the healthy ones ($p \leq 0.0006$ and $p \leq 0.02$, respectively). Besides, most inflammatory cytokines were up regulated, while anti-inflammatory cytokines were down regulated in the PE placentas. Additionally, TNF-α/IL-35, IFN-γ/IL-35, IL-6/IL-35, and IL-23/IL-35 ratios were significantly higher ($p \leq 0.01$) and IL-35/IL-17 ratio was significantly lower ($p \leq 0.05$) in the preeclamptic patients compared to the healthy controls.

Conclusion: The results indicated that Th1/Th2/Th17/ Treg balance within placenta determined the fate of a normal pregnancy. Moreover, regulatory T cells seemed to play a central role in regulation of all subsets. This study also found and highlighted a new regulatory cytokine, IL-35, for balancing T helper responses in placenta.

Key words: *Preeclampsia, T helper, Transcription factor, Cytokines, IL-35, Real-time PCR.*

O-41
MSC administration improves murine pregnancy outcome in abortion-prone mouse model with involvement of CD80/86 and CD28/CTLA-4

Salek Farrokhi A[1], Zarnani AH[1], Moazzeni SM[2].

1. *Department of Immunology, Tarbiat Modares University, Tehran, Iran.*
2. *Nanobiotechnology Research Center, Avicenna Research Institute, Tehran, Iran.*

Email: A.salek@modares.ac.ir

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy with a prevalence of 2-5% among pregnant women. Immune regulation during pregnancy is complex, and thus an optimal therapy for pregnancy complications is always a big challenge to reproductive medicine. It seems that mesenchymal stem cells may improve the immunological condition in immune mediated RSA and help to maintain the fetus.

Materials and Methods: The MSCs were derived from the abdominal fat of CBA/J mice. On the day 4.5 of gestation MSCs was administered (i.p) to mice in the test group. On day 13.5 of pregnancy, abortion rates were calculated and CD80, CD86, CD28 and CTLA-4 gene expression in the decidua and placenta was evaluated by Real-Time PCR.

Results: The MSC group presented significantly diminished abortion rates as compared to abortion group, as expected (5% vs. 29.83%, p=0.0045). It was demonstrated that administration of MSCs at the window of implantation significantly up-regulated the expression of CTLA-4, while down-regulating the levels of CD80, CD86, and CD28 at the fetal maternal interface.

Conclusion: In this study, we showed that modulation of costimulatory molecule expression by MSCs administration might contribute to preventing the fetus from maternal immune attack. Together, these findings indicate that MSCs has a beneficial effect on the fetal maternal interface in abortion prone mouse model, leading to a pregnancy outcome improvement, which might provide new therapeutics for spontaneous pregnancy loss.

Key words: *Recurrent spontaneous abortion, Mesenchymal stem cell, Immunological condition, Costimulatory, Co-inhibitory.*

O-42
Effects of nulliparity and multiparity on breast cancer

Ghasemi N[1], Hajiaghayi M[1], Rezayat F[1], Mesdaghi M[1], Ramezani Tehrani F[2], Mosaffa N[1].

1. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: nazanin.ghasemi69@gmail.com

Introduction: According to the Microchimerism hypothesis, the relation between lower incidence of breast cancer and multiparity has been controversial.

Materials and Methods: Peripheral blood mononuclear cells of 48 multiparous and nulliparous women was isolated. Cell proliferation and percentage of CD3+ CD8+ lymphocytes in two-time (48 and 72 hours) after co-culture of breast tumor cell lines (MDA-231 and MCF-7) with PBMC measured by Brdu Assay and Flow cytometric analysis.The level of TNF-a concentration was detected by ELISA technique.

Results: Effector cells proliferative response in co-culture of MCF-7 is more than MDA-231 and between multiparous and nulliparous women 48h after co-culture in both cell lines, was significant ($p \leq 0.001$). The mean of lymphocyte proliferation 72h after co-culture was statistically significant ($p \leq 0.001$). It also determines the percentage of the population of cytotoxic lymphocytes (CD3+ CD8+) showed no significant difference between the two groups. TNF-a was significant rises in multiparous samples.

Conclusion: Increasing the lymphocyte proliferation during co-culture, shows that multiple pregnancy can provoke anti-tumor response and resistance to the development of cancer. However, the conclusion about relationship between breast cancer and parity requires an examination of additional anti-tumor responses with higher sample volume.

Key words: *Breast cancer, Nulliparity, Multiparity.*

O-43

Follicular fluid IL-17 and IL-23 in patients who were at the risk of OHSS and cumulus gene expression during IVF/ET cycles

Motamedzadeh L[1, 2], Hadinedoushan H[3], Farashahi Yazd E[1], Mohammadi MM[2, 4].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Immunology and Allergy, Kerman University of Medical Sciences, Kerman, Iran.*
3. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran.*

Email: lmotamedzadeh5858@gmail.com

Introduction: Ovarian Hyper Stimulation Syndrome (OHSS) is one of the major complications during assisted reproductive technology. The role of cytokines in the pathology of OHSS has been interesting research field in the last years.

Materials and Methods: Forty OHSS women seeking for IVF were included in this cross sectional study. Also some female patients with male factor infertility seeking for IVF were selected as the control group (n=40). Controlled ovarian stimulation was performed with antagonist protocol. The ovarian puncture was performed for all patients. The follicular fluid concentration of IL-17 and IL-23 was determined by ELISA in both groups. The gene expression of IL-17 and IL-23 in cumulus cells was compared between two groups by qPCR as well. Serum E2, FSH, LH, AMH, PRL, and anti-TPO were investigated for all patients. The number of mature oocytes was evaluated in both groups.

Results: OHSS patients had higher follicular fluid IL-17A (4.32±1.5 pg/ml) than the control group (3.72±1.1 pg/ml). But the IL-23 was the same between OHSS group (59.24±3.6) and the control group (54.3±3.1). The gene expression showed no significant differences for IL-17A and IL-23 between the two groups. There was a positive significant correlation between the number of MII oocytes and IL-23 in OHSS cases. The concentration of E2 and AMH were significantly higher in OHSS patients compared to controls ($p \leq 0.0001$), but the LH, FSH, TSH, PRL, and Anti-TPO levels were similar between two groups. The rate of MII oocytes, fertilized oocytes, and good embryo formation for transfer were significantly higher in OHSS patients compared to controls.

Conclusion: IL17 in FF of patients with OHSS is significantly higher than that was found in controls. To the best of our knowledge, this is the first report of IL17 assessment in FF in OHSS cases.

Key words: *OHSS, IL-23, IL-17A, ELISA, qPCR, FF, Gene Expresion.*

O-44

The effect of active form of 1,25 VitD3 on T regulatory cells in patients with Recurrent Spontaneous Abortion (RSA)

Abdollahi E, Vahidi Z.

Department of Immunology, Immunology Research Center, BuAli Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Email: ea6112@gmail.com

Introduction: Recurrent spontaneous abortion (RSA), defined as three or more consecutive pregnancy losses before the 20th week of gestation, occurs in 1-5% of women of reproductive age. CD4+ CD25+ CD127- FoxP3+ Treg cells constitute a minority of the CD4+ T cell population in peripheral blood cells. In the normal pregnancy, Tregs prevent the generation of an immune response against fetal tissue and a decrease in the number of Tregs is associated with abortion. 1,25VitD3 acts directly on T cells to promote FoxP3+ and IL-10+ Tregs, secretion of the immunomodulatory cytokines IL-10 and transforming growth factor (TGF)-β.

Materials and Methods: Ten patients with RSA were sampled for 10 ml whole blood to isolate peripheral blood mononuclear cells (PBMCs) using Ficoll-Hypaque density gradient centrifugation. Isolated cells were cultured in the presence of 50 nM 1,25VitD3. Treg cells were analyzed by flowcytometry after and before treatment with 1,25VitD3.

Results: There was a significant difference between the percentage of CD4+ CD25 bright CD127- T cells before and after treatment with vitamin D (0.59% vs. 1.24% $p<0.05$).

Conclusion: This study showed that 1,25 VitD3 increases Treg percentage in patients with RSA and revealed that this metabolite can exert as a supplementary therapeutic in patients with RSA.

Key words: *RSA, T Regulatory cells, 1,25 Vit D3.*

O-45

MSC administration can prevent over percipitation of C3 complement in the decidua and placenta of abortion prone mice

Shahgaldi Sh, Moazzeni SM.

Department of Immunology, Tarbiat Modares University, Tehran, Iran.

Email: shahab.shahgaldi@modares.ac.ir

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy with a prevalence of 2-5% among pregnant women. Immunological disorders are one of the main causes of recurrent spontaneous abortions (RSA). Complement activation is involved in the development of miscarriages and has emerged as a common event in recurrent pregnancy loss. Mesenchymal stem cells (MSCs) have been shown to modulate immune responses and reduce the abortion rate following MSC therapy.

Materials and Methods: MSCs were derived from abdominal fat (AT-MSCs) of CBA/J mice. On the 4.5th day of gestation, the test group (CBA/J ×DBA/2) received an IP injection of 1×10^6 of AT-MSCs while the control (CBA/J × DBA/2) and normal pregnancy (CBA/J × BALB/c) groups recieved an IP injection of PBS. On the 13.5th day of gestation, tissue samples (placentae and deciduae of pregnant mice) were analyzed to measure complement C3 deposition using immunohistochemistry.

Results: As expected, the resorption rate was significantly lower in the test group as compared to the control group. Moreover, the data obtained from immunohistochemical analysis demonstrated that complement C3 deposition remarkably decreased in the placenta and decidua of abortion prone mice upon MSC administration.

Conclusion: Here, we showed for the first time that low levels of complement C3 deposition induced by MSCs administration reduced abortion rate in the abortion prone mouse model. Our results suggested that MSCs could induce their immunomodulatory effects through decreasing complement C3 deposition, which leads to decrease in abortion rate.

Key words: *Mesenchymal stem cell, Recurrent spontaneous abortion, Cell therapy, Complement deposition.*

O-46

Intravenous immunoglobulin (IVIG) modulates regulatory T cells and improves live birth rate in women with recurrent spontaneous abortion

Ahmadi M[1], Dolati S[1], Nouri M[2], Babaloo Z[1], Farzadi L[3], Yousefi M[1].

1. *Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Department of Reproductive Biology, Tabriz University of Medical Sciences, Tabriz, Iran.*

Email: m.ahmadi6892@gmail.com

Introduction: Recurrent spontaneous abortion (RSA) is defined as three or more repeated abortions, can be caused by maternal immunological rejection. Treg cells are recently proposed as new risk factors in RSA. IVIG therapy for RSA patients began in the late 1980s, indicated for the women with miscarriages associated with antiphospholipid antibodies (APA). IVIG therapy was then recommended for patients with recurrent spontaneous abortion. However, the molecular and cellular mechanisms underlying IVIG effects on the prevention of abortions are not completely understood.

Materials and Methods: In total 38 women with RSA with cellular immune abnormalities were included and peripheral blood was drawn upon positive pregnancy test. On the same date, IVIG, 400 mg/kg, was administrated intravenously and continue every 4 weeks through 28-30 wks of gestation. For control, 12 RSA patients with abnormal cellular immune profile were included as IVIG untreated group. We investigated IVIG effect on Treg cells frequencies and cytokine secretions and pregnancy outcome in RSA patients before and after treatment.

Results: Treg cells was increase from 3.55±1.65 to 9.13±1.23 in IVIG treated group. Moreover, significant increase of Foxp3, IL10 and TGF-β mRNAs and protein secretions were observed in IVIG treated patients. Pregnancy outcome in IVIG treated subjects (82.4%) was significantly higher than untreated group (41.6%).

Conclusion: Our findings suggest that the mode of action of IVIG in the prevention of immunological abortions particularly in those with cellular immune abnormalities may be through a shift in T cell differentiation in favor of the Treg-type response.

Key words: *Recurrent spontaneous abortion, Intravenous immunoglobulin G, Treatment, Treg.*

Poster Presentations

7th Yazd International Congress and Student Award in Reproductive Medicine

P-1
The role of TSLP in pathogenesis of endometriosis

Habibi S[1, 2], Poursadoughian Yaran A[2, 3], Afsharian P[2], Shahhoseini M[2], Amirchaghmaghi E[4, 5], Aflatoonian R[5], Naji T[2], Ramazanali F[5].

1. *Faculty of Basic Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran.*
4. *Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
5. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: habibi.soolmaz@yahoo.com

Introduction: Endometriosis is defined as growth of endometrial-like tissue outside the uterus. It has been suggested that immunological factors play roles in the pathogenesis of endometriosis. Thymic stromal lymphopoietin TSLP is one of important cytokines. TSLP Receptor, also known as cytokine receptor-like Factor 2 (CRLF2) forms a functional heterodimeric complex with IL-7R to bind with TSLP. Twenty three polymorphisms have been reported for *TSLP* gene that one of them (C/T) is located on promoter region (-847) (rs3806933).

Materials and Methods: This study consisted of two groups (women with endometriosis and healthy fertile women). Endometrial and blood samples were obtained from each group. Ectopic endometrial biopsies (n=15) were collected through laparoscopic procedure while eutopic endometrial tissues (n=15) and endometrial biopsies of controls (n=16) were obtained by pipelle. The expression level of TSLP and *TSLPR* genes were evaluated by using quantitative polymerase chain reaction (Q PCR). C-847 T polymorphism was studied in 100 blood samples of each group. *TSLP* promoter SNP (rs3806933) was genotyped using the PCR followed by direct Sanger sequencing.

Results: QPCR analysis showed that the mRNA expression levels of TSLP and TSLPR chains (CRLF2, IL7Rα) were significantly increased in ectopic tissues of patients with endometriosis compared to eutopic and normal endometrial tissues. Promoter polymorphism study was resulted in observation of CC, CT and TT genotypes in 9%, 61% and 30% of endometriosis group whereas their frequencies were 16%, 44% and 40% in control group, respectively.

Conclusion: Correlation between C-847T polymorphism and elevated of expression of TSLP was significantly with susceptibility to endometriosis.

Key words: *Endometriosis, Immune response, Thymic Stromal Lymphopoietin (TSLP), Polymorphism.*

P-2
mRNA expression of DNA methyltransferases and the effect on global methylation and P1 to P2 ratio in patients with severe sperm abnormalities

Rahiminia T, Talebi AR, Farashahi Yazd E, Ghasemi-Esmailabad S.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: tahereh.rahiminia@gmail.com

Introduction: Oligoasthenoteratospermia is a sever infertility condition in men which can be related to epigenetics disorders. DNA methyltransferase1, 3A and 3B (DNMTs) are responsible for adding methyl groups to carbon 5 position of cytosines located at CpG dinucleotides to establish the DNA methylation. Alteration in sperm global methylation can disregulate the gene exprerssion like protamine1 (P1) or protamine 2 (P2) as the main proteins in sperm chromatin compaction result changes in P1 to P2 mRNA ratio in sperm of oligoasthenoteratospermic (OAT) patients.

Materials and Methods: In this prospective study, processed sperm by density gradient method was compared in 30 AOT patients and 30 cases of normozoospermia by using real-time quantitative reverse transcriptase polymerase chain reaction for mRNA expression of DNMT1, DNMT3A, DNMT3B, P1 and P2; enzyme-linked immunosorbent assay for global methylation. Sperm analysis was done according to strict criteria. Nonparametric variables were calculated using the Mann-Whitney U test, Kruskal-Wallis H test and spearman method. Statistical significance was considered at $p \leq 0.05$ using SPSS software, version 18.

Results: Increasing level of DNMT3A, DNMT3B mRNA expression and decreasing of P1 to P2 ratio and a high level of sperm global methylation significantly occured in OAT patients compared to normozoospermic men. DNMT1 was correlated to DNMT3A, DNMT3B mRNA expression and global methylation but not to P1 to P2 ratio.

Conclusion: Different altered mRNA expression levels of DNMT1, DNMT3A and DNMT3B did not affect global methylation and P1 to P2 mRNA ratio of sperm in OAT patients.

Key words: *DNA methyltransferase, P1 to P2 ratio, Global methylation.*

P-3

A comparative study of dydrogesterone and micronized progesterone for luteal phase support during in vitro fertilization (IVF) cycles

Saharkhiz N[1], Zamaniyan M[2], Salehpour S[1], Zadehmodarres Sh[1], Hoseini S[1], Cheraghi L[3], Seif S[4], Baheiraei N[5].

1. *Preventive Gynecology Research Center (PGRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Infertility Center, Department of Obstetrics and Gynecology, Mazandaran University of Medical Sciences, Sari, Iran.*
3. *Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
4. *Department of Veterinary Theriogenology and Obstetric, Tehran University of Medical Sciences, Tehran, Iran.*
5. *Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: marziehzamaniyan@gmail.com

Introduction: The aim of the present study was to compare the efficacy, tolerability and patients' satisfaction after the use of oral dydrogesterone with vaginal micronized progesterone for luteal-phase support (LPS) among infertile women undergoing in vitro fertilization (IVF).

Materials and Methods: A total of 210 women (aged 20-40 years old) with a history of infertility, who underwent controlled ovarian stimulation for fresh intra-cytoplasmic sperm injection-embryo transfer cycles, were included in the study. Consequently, they were randomized to receive LPS with dydrogesterone 20mg twice daily (n=96) or micronized progesterone 400mg twice daily at the day of oocyte retrieval (n=114).

Results: The clinical success rate (31% vs. 33%; p=0.888), miscarriage rate (5.0% vs. 3.0%; p=0.721), ongoing pregnancy rate (30.0% vs. 30.0%; p≤1.000), implantation (22.0% vs. 24.0%; p=0.254) and multiple pregnancy rate (5.30% vs. 7.20%; p=0.394) were comparable among the two groups. Serum progesterone levels were significantly lower among the patients receiving dydrogesterone than the control group (13.62±13.83 ng/ml vs. 20.66±18.09 ng/ml; p≤0.001). However, there was no statistically significant difference regarding the patients' satisfaction (p=0.825) and tolerability (0.790) between the two groups.

Conclusion: Our results showed that oral dydrogesterone (40 mg/day) is as effective as vaginal micronized progesterone considering its clinical outcomes and patients' satisfaction and tolerability, for LPS among women undergoing IVF.

Key words: *Dydrogesterone, In vitro fertilization, Luteal-phase support, Micronized progesterone, Progesterone.*

P-4

Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in normal infertile women

Hosseinisadat R.

Department of Obstetrics and Gynecology, Kerman University of Medical Sciences, Kerman, Iran.

Email: robabehosseinisadat@yahoo.com

Introduction: Despite major advances in assisted reproductive techniques (ART), the implantation rates remain relatively low. One of the options in studies that rising implantation is intrauterine infusion of granulocyte colony stimulating factor (G-CSF).

Materials and Methods: This study was a randomized controlled clinical trial that 113 infertile women with normal endometrial thickness who were candidate for IVF were participated in two groups. Our exclusion criteria were history of repeated implantation failure (RIF), endocrine disorders, severe endometriosis, congenital or acquired uterine anomaly and contraindication for G-CSF (renal disease, sickle cell disease, or malignancy). In case group (n=55) 300µg trans cervical intrauterine G-CSF was administered in oocyte retrieval day. Controls (n=58) were treated with standard protocol. Main outcomes include chemical, clinical and ongoing pregnancy rates, implantation rates, and miscarriage rates were measured in two groups.

Results: There were not statistically differences in chemical, clinical and ongoing pregnancy, implantation rate, and miscarriage rate between two groups.

Conclusion: In normal IVF patients, G-CSF does not affect chemical and clinical pregnancy rates, implantation rates and miscarriage rates.

Key words: *G-CSF, Granulocyte colony-stimulating factor, In vitro fertilization, Pregnancy rates, Randomized controlled trial.*

P-5

Assessment of serum level of CXCL5 cytokine in women with Polycystic Ovary Syndrome (PCOS) with normal BMI: A cross-sectional study

Zohrabi M[1], Rahmani E[2], Motamed N[3], Akbarzadeh S[4].

1. *The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran.*
2. *Department of Obstetrics and Gynecology, Bushehr University of Medical Sciences, Bushehr, Iran.*
3. *Department of Community Medicine, Bushehr University of Medical Sciences, Bushehr, Iran.*
4. *Department of Biochemistry, Bushehr University of Medical Sciences, Bushehr, Iran.*

Email: zohrabimarzieh@yahoo.com

Introduction: Polycystic Ovary Syndrome (PCOS) is the most common endocrine disease. CXCL5 is a new cytokine secreted by adipose tissue that inhibits the activity of insulin in muscles and promotes insulin resistance.

Materials and Methods: This research is a cross-sectional case control study that thirty women with PCOS based on the Rotterdam 2003 diagnostic criteria were chosen as case and thirty women with normal menstrual cycles and no signs of infertility were selected as control. Serum levels of insulin and CXCL5 were measured with ELISA method. Data were analyzed using SPSS software version 16.

Results: The results from this study show a significant increase in the concentrations of insulin, HOMA-IR,

LH, LH/FSH, FBS, testosterone and prolactin, while no significant differences were seen in CXCL5, cholesterol, LDL-C, HDL-C, Creatinine and HOMA-B in the case group compared with control group.
Conclusion: Since serum concentrations of CXCL5 increases in obesity and weight gain, probably it can be stated that CXCL5 in obese PCOS women can be effective in causing metabolic disorders. There was no significant changes in serum concentrations of CXCL5 in non-obese PCOS women. So we can conclude that the metabolic disorders in PCOS women may increase with weight gain via changes of CXCL5 concentration.
Key words: *Polycystic Ovary Syndrome, Insulin resistance, CXCL5 Chemokine, Hyperinsulinemia, Hyperandrogenism.*

P-6
The effect of Citrullus colocynthis extract on inflammatory and oxidative stress in estradiol valerate- induced Polycystic Ovarian Syndrome in rat

Barzegar MH[1], Khazali H[1], Kalantar SM[2], Khoradmehr A[2].
1. *Department of Physiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: homayoun_khazai@yahoo.com

Introduction: Polycystic ovarian syndrome (PCOS) is a reproductive and metabolic disorder which the level of gonadotropins and sexual hormones in blood are imbalanced. These disorders can be related to oxidative stress and chronic low grade inflammatory state. The goal of this study was to determine whether *CCT* extract affects the mean plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, glucose and function and structure of ovary in EV-induced PCOS rats.
Materials and Methods: Forty Wistar rats female were selected (160-220 gr). They had every 2-3 consecutive estrous cycle during 12 to 14 days. The first two groups were divided into control (n=8) and polycystic (n=32). PCOS were induced by estradiol valerate injection after 60 days. The polycystic groups were divided into four groups (n=8 in each group). PCOS group and three experimental groups, which were orally received 50 mg/kg metformin, 50 mg/kg *Citrullus colocynthis* extract and 50 mg/kg metformin+50mg/kg *Citrullus colocynthis* extract for 20 days. The serum level of FSH, LH and testosterone were measured using ELISA method, while the serum concentrations of glucose were measured using oxidative methods. Histological study on the liver tissue for evaluation toxic effects and histomorphological study on the ovary carried out by Hematoxylin and Eosin (H&E) method. The data analyzed using the one-way ANOVA method considering $p<0.05$ level of significance.
Results: There was a significant reduction in LH and testosterone in extract treated groups compare to PCOS. But the serum level of FSH showed no significant change in treatment groups compare to PCOS. Moreover, histomorphometric studies also showed the significant changes in the number of pre antral and antral follicles. No histopathological changes was seen in liver tissue with administrated dose of extract (50 mg/kg).
Conclusion: These results demonstrated a marked improvement in the symptoms of PCOS which may be due to *Citrullus colocynthis* effects on inflammation and oxidative stress pathways. Hence this plant can be considered as a potentially effective drug for treatment of PCOS.
Key words: *Polycystic ovary syndrome, Wistar rat, Citrullus colocynthis, Inflammation.*

P-7
Cytogenetic studies on couples experience recurrent pregnancy loss referring to Mehr Infertility Clinic in Rasht

Fazel Tolami H, Mehrafza M, Askari M.
Department of Genetic, Mehr Medical Institute, Rasht, Iran.
Email: hf.tolami@gmail.com

Introduction: Recurrent spontaneous abortion is losing two or more pregnancy before week 20 due to infection, genetic, hormonal, anatomic and immunologic disorders. Studies have shown that the parents chromosomal abnormalities could be among the reasons for the occurrence of recurrent abortion and infertility.
Materials and Methods: This study have been implemented on 64 couples who have a history of 2 times or more spontaneous recurrent abortion. After reviewing medical records, genetic counseling and drawing family genealogy, the standard 72 hr lymphocytic culture test and CTG-Banding karyotype have been performed.
Results: Data analysis has shown the chromosomal abnormalities in 12.2% of the cases. Almost 46 percent of these abnormalities are related to women and the rest to men. Nearly half of anomalies were numerical and others were structural. finally, 54% of abnormalities located on sex-chromosomes while others were related to autosomal chromosomes.
Conclusion: The chromosomal abnormalities in parents can be one of the major reasons in spontaneous pregnancy loss and recurrent abortions. Therefore, the genetic counseling and, if necessary the cytogenetic tests in parents can be a good way to understand the reasons of abortion or prevent the birth of children with chromosomal disorders.
Key words: *Chromosomal abnormalities, Recurrent abortion, Karyotype, CTG-banding.*

P-8
Effects of Phenobarbital administation in third to sixth days of pregnancy on uterus of Balb/C adult female mouse

Saffari S, Torabzadeh P, Monfaredi M.

Department of Bioology, Karaj Branch, Islamic Azad University, Karaj, Iran.
Email: safoorasaffari66@gmail.com

Introduction: Phenobarbital is a tranquilizer drugs and barbiturate drugs component which can be used in the treatment of epilepsy.
Materials and Methods: At the first a lethaldose LD50 was determined in condition of 3.48 ml/kg bw in vivo. Experience with respect to a threshold dose was continued with selected dose of 1.20 ml/kg.bw. Injection of Phenobarbital was done on the third to sixth days by enema. On the fifteenth day of pregnancy, mice were autopsied and uterus was separated. The results experimental groups (eache groups:10 mice) were compared with the control group (non-injection: 10 mice) and sham (injection of normal saline:10 mice). Data was checked with SPSS 20 software and Duncan post test, subject to $p \leq 0.001$ and $p \leq 0.05$.
Results: Macroscopic and microscopic studies showed that Phenobarbital injection causes swelling of the uterus in mice. The inflation percentage was 60% in the third and fourth day of pregnancy, while it was 50% in the fifth day of pregnancy and 30% in the sixth day of pregnancy.
Conclusion: Our results indicated that probably Phenobarbital have impact on sex hormones and can cause miscarriage or infertility. Therefore in controlling epileptic seizures high doses of this drug in early days pregnancy should be avoided.
Key words: *Phenobarbital, Epilepsy, Uterus, Infertility.*

P-9
Effect of oocyte vitrification on mitochondrial gene expression

Allahveisi A[1], Rezaie MJ[1], Nikkhoo B[2], Yousefian E[3].
1. *Infertility Center of Besat Hospital, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
2. *Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
3. *Department of Obstetrics and Gynecology, Infertility Center, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: allavaisie@gmail.com

Introduction: Oocyte vitrification is an important technique available to fertility presevation of patient women with premature ovarian failure (POF) after chemotherapy or radiotherapy. Results from post-thawed metaphase II (MII) human oocytes revealed low fertilization and implantation rates. The causes of poor results from thawed human oocytes are unclear. Mitochondria provide main energy of the oocyte by synthesize ATP, which is the essential factor for fertilization and early embryo development. The mature human oocytes have about 100 000 numbers of mitochondria as well as mitochondrial DNA (mtdna) copy number. Mitochondrial transcription factor A (TFAM) is mobility group proteins which essential for maintenance and pakage of mitochondrial DNA (mtdna). The aim of this study was to investigate the effect of vitrification on mitochondrial transcription factor a (TFAM) of metaphase II (MII) human oocytes.
Materials and Methods: oocytes were obtained from healthy women undergoing ICSI /IVF because of male factor infertility between19 and 37 years of age. The total oocytes were divided into two controls and experimental group. All oocytes of experimental groups were vitrified using vitrification kit and were kept in liquid nitrogen. The mRNA of TFAM after thawed-oocytes was measured using qPCR.
Results: Our studies showed that expression level of transcript mitochondria TFAM following vitrification of metaphase oocyte decreased as compared with that fresh of metaphase II oocytes $p<0.05$.
Conclusion: the alternative of mitochondrial transcript after the freezing/thawing oocytes may be cause of poor oocyte quality.
Key words: *Oocyte quality, Vitrification, Mitochondria.*

P-10
Comparison of antisperm antibody and DNA fragmentation sperm in patients with idiopathic oligoasthenoteratspermia

Allahveisi A[1], Rezaie MJ[2], Nikkhoo B[3], Yousefian E[4].
1. *Infertility Center of Besat Hospital,Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
2. *Deparment of Pathology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
3. *Department of Obstetrics and Gynecology, Infertility Center, Isfahan University of Medical Sciences, Isfahan, Iran.*
4. *Department of Obstetrics and Gynecology, Isfahan University of Medical Sciences, Isfahan.*

Email: allavaisie@gmail.com

Introduction: Infertility is a public health problem which affects about 10-15%. The male factor is reportedly up to 50% of infertile couples. Multiple causes result in male infertility including impair of anatomy, physiology, endocrinology or genetics. Oligoasthenoteratozoospermia (OAT) is most severe abnormalities of sperm parameters which associated with low sperm count, poor motility and high abnormality sperm morphology. Previous reported that the immune system activation may be associated with idiopathic OAT. However, sperm autoimmunity in etiology of OAT is not clearly understood. The aim of the present study was to determine antisperm antibody (ASA) in infertile men with OAT.
Materials and Methods: Infertile men with OAT aged 26-41 yr undergoing infertility treatment. Semen samples were selected according to the WHO, 2010 criteria described by (sperm concentration $<15\times10^6$, progressive motility <32%; normal forms <4%). SCD kit used to evaluate DNA fragmentation. The direct immunobead test was used to assay the ASA level of sperm samples.
Results: Sperm concentration and motility were significantly lower in ASA-positive as compared to

DNA fragmentation ($p<0.05$). Sperm normal forms lower in DNA fragmentation. However, morphology sperm were not significantly in ASA-positive as compared to DNA fragmentation ($p<0.05$).

Conclusion: Current study showed that ASA are associated with sperm motility and sperm concentration. DNA fragmentation is correlated to abnormal morphology sperms.

Key words: *DNA fragmentation, Oligoasthenoteratspermia, Antisperm antibody.*

P-11

Effects of Lavendula officinalis aqueous extract on fertility and embryo of Balb/C mouse

Soheili F[1], Torabzadeh P[2], Saffari S[1].

1. *Department of Biology, Karaj Branch, Islamic Azad University, Karaj, Iran.*
2. *Department of Biology, Karaj Branch, Islamic Azad University, Karaj, Iran.*

Email: soheilifatemeh@yahoo.com

Introduction: In addition to the sedative properties of Lavendula officinalis aqueous extract, it has antimicrobial properties. However, its effect on fertility has not been reviewed yet.

Materials and Methods: After preparation of aqueous extract, experiments was done on 65 mice with selected doses of; 6 (group 1: 15 mice), 12 (group 2: 15 mice) and 18 (group 3: 15 mice) g/kg bw. Interperitoneally injections were done for 12 days. Results were compared with the control group (non-injection) and sham (injection of normal saline). For reliability of the results, experiences were repeated 3 times. Data was checked with SPSS 20 software and Duncan post test and ANOVA subject to ($p<0.001$) and ($p<0.05$).

Results: According to the results, the experimental group 1 was pregnant earlier than the other groups and significant decrease was seen in the number of mouse pups in group 2 and 3 ($p<0.001$ and $p<0.05$). In group1 all mouse pups were healthy but we saw a significant increase ($p<0.001$) of abnormal embryos in group 2 and 3. There was a large number of mouse pups in group 2 with bleeding eyes, head and skull, also limb deviation from the symmetry axis and extensive bleeding in the whole body was observed in group 3. Finaly all experimental groups showed a significant decrease of body weight ($p<0.05$).

Conclusion: It can be stated that consumption of high doses Lavendula officinalis cause impairment of fertility and embryo abnormalities.

Key words: *Lavendula officinalis, Fertility, Abnormalities, Embryo mouse.*

P-12

Fertilization rate following PESA-ICSI and TESE-ICSI: an eight years retrospective cohort study in Yazd

Ghasemi-Esmailabad S, Borzouie Z, Moshrefi M, Moein MR.

Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: qasemisaeed@yahoo.com

Introduction: The prevalence of male infertility due to azoospermia [obstructive (OA) and non-obstructive (non OA)] is about 10%. The treatment approaches, suggested for these patients, compromised of sperm retrieval from testis or epididymis.

Materials and Methods: A retrospective 8 yr results of PESA-ICSI and TESE-ICSI cycles, performing from 2008-2016 in Yazd research and clinical center for infertility, were collected. We studied the records of 764 men with obstructive and non-obstructive azoospermia who underwent sperm retrieval for intracytoplasmic sperm injection. 658 cycles of TESE and 106 cases of PESA, were considered in this study. Also patients' demographic information was collected. The fertilization rate was recorded and the data were analyzed using SPSS software.

Results: The mean value of age was 33.34 yr. Our results show that 93 cycles of PESA were positive whereas 13 cycles were negative. Also, 156 cycle of TESE were negative while 502 cycles were positive. The rate of fertilization was higher in TESE group.

Conclusion: On the base of our results, ICSI cycle is more effective with testicular sperm than sperm retrieval from epididymis irrespective the cause of azoospermia. The chance of fertilization rate was higher in TESE-ICSI surgery.

Key words: *ICSI, Percutaneus sperm aspiration, Testicular sperm extraction, Fertilization rate.*

P-13

The effect of Tribulus terrestris extract on motility and viability of human sperm after cryopreservation

Khaleghi S[1], Bakhtiyari M[2], Asadmobini A[1], Esmaeili F[3].

1. *Department of Physiology, Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
2. *Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
3. *Infertility Treatment Research Center, Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

Email: sarakhaleqi2014@gmail.com

Introduction: Semen cryopreservation produces significant amounts of reactive oxygen species (ROS), which may lead to impairment of sperm morphology, function, and ultimately, male fertility.

Materials and Methods: Semen specimens from 80 healthy volunteers were divided into eight groups: fresh control (group I), freeze control (group II), groups III, IV, and V, which had 20, 40, and 50 µg/mL doses of Tribulus terrestris extract added before cryopreservation, and groups VI, VII, and VIII, which were supplemented by these extract doses after the freeze-thaw process. To evaluate the effects of the

Tribulus terrestris extract, the semen samples were incubated with the extract and evaluated with a light microscope for motility and viability.

Results: After cryopreservation, a significant improvement in spermatozoa viability was observed in group VII. In groups VII and VIII, motility, according to World Health Organization (WHO) criteria, increased considerably ($p \leq 0.001$). There was no significant difference among groups III, IV, and V.

Conclusion: The present study demonstrated that the protective effects of Tribulus terrestris, which improves human sperm motility and viability, may be due to its antioxidant properties. On the basis of the results, the researchers concluded that Tribulus terrestris can be used as a safe therapeutic alternative to current modalities for the management of motility dysfunction in males.

Key words: *Tribulus terrestris, Human sperm, Motility, Viability, Cryopreservation, Reactive oxygen specie.*

P-14
Protamine sequence variants in Iranian infertile men with idiopathic teratozoospermia

Dehghanpour F[1], Fesahat F[1], Miresmaeili SM[1], Zareh Mehrjardi E[2], Talebi AR[1].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*

Email: dehghanpour64@yahoo.com

Introduction: Single nucleotide polymorphism (SNPs) are considered as one of the underlying reason of male infertility. Abnormal protamine replacements have been indicated to cause DNA damage and infertility.

Materials and Methods: In this study, several SNPs in *PRM1* (c.49 C>T, c.102 G>T and c.230A>C) and *PRM2* (rs545828790, rs115686767, rs201933708, rs2070923 and rs1646022) genes were investigated in 27 idiopathic infertile men with teratospermia in comparison to 25 normal control men. Analysis of SNPs was performed using PCR-direct sequencing.

Results: In *PRM1* gene c.230A>C, as a synomynous polymorphism, was detected in teratozoospermic men (heterozygous n=26; homozygous n=1) and controls (heterozygous n=15; homozygous n=4). All patients and control groups were determined normal for a missense mutation of rs545828790 in *PRM2* as well as rs115686767 and rs201933708, as a synonymous mutation. The findings showed an intronic variant of rs2070923 in *PRM2* gene among two groups. Also, rs1646022, as a missense polymorphism, occurred in teratozoospermic men (heterozygous n=10; homozygous n=5) and controls (heterozygous n=13; homozygous n=2). However, there were no significant differences in evaluated SNPs of *PRM1* and *PRM2* between patients and control groups, except for c.230A>C that frequency of altered CA genotype was significant in infertile men with teratozoospermia (p=0.001).

Conclusion: It was demonstrated that evaluated SNPs do not have any devastating impact on expression of the protamine proteins in infertile men with teratozoospermia.

Key words: *Single nucleotide polymorphism, Teratozoospermia, Protamin, Sperm.*

P-15
The perception and experience of infertile women undergoing IVF/ICSI about acupressure: A qualitative study

Khadivzadeh T[1], Hassanzadeh Bashtian M[1], Badiee Aval Sh[2], Esmaily H[3].

1. *Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Acupuncture, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *Department of Biostatistics and Epidemiology, Mashhad University of Medical Sciences, Mashhad, Iran.*

Email: khadivzadeht@mums.ac.ir

Introduction: Fertility in many cultures has a high value and having a child is one of the most fundamental human stimuli that if it fails, can become a destructive emotional experience. In this crisis, infertile couples are more than other people affected by depression, anxiety, low self-esteem and dissatisfaction.

Materials and Methods: This study was a qualitative study using conventional content analysis which performed on 16 infertile women undergoing IVF/ ICSI in Milad IVF Center, Imam Reza Hospital, Mashhad University of Medical Sciences. Qualified individuals purposefully were selected according to inclusion and exclusion criteria and were randomly divided into two groups of real and sham acupressure group. In the real acupressure group P6 and HT7 points and in the sham acupressure group points with two centimeters away from the main points on two hands were under acupressure. Acupressure was performed in twelve sessions, four sessions by the investigator and eight sessions by own patient. The patient was trained by investigator. After the intervention participants were interviewed. Then, interviews were organized and coded. Data analysis by using of conventional content analysis based on three primary phases of preparation, organization and reporting was done.

Results: Results obtained from experience and perception were in two categories; body understandings and positive experiences from acupressure. The results show that perceived acupressure by infertile women is effective on their health.

Conclusion: According to results obtained from this study, it would be better designing one questionnaire based on qualitative results, also more studies be done about social flexibility and individual in women related to reproductive and sexuality issues.

Key words: *Acupressure, Qualitative, IVF/ICSI, Infertile women.*

P-16
Doing an active role of mothers in delivery: A grounded theory study

Mirzaee F[1], Mirzaii Najmabadi Kh[2], Taghipour A[3].
1. *Razi School of Nursing and Midwifery, Kerman University of Medical Sciences, Kerman, Iran.*
2. *School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*

Email: f_mirzaee@kmu.ac.ir

Introduction: Feeling of have an active role of mothers in delivery induces to have a positive experience of childbirth in women. The involvement of mothers in decision making in vaginal childbirth is main factor of women's autonomy.
Materials and Methods: In this grounded theory, between March 2013 to April 2014, 29 participants including women who had vaginal delivery, midwives and obstetrician were selected. Semi- structured interviews and observations were conducted for data collection. Open, axial and selective coding was used for data analysis. Maxqda software (version, 2007) was also used in data analysis.
Results: The core category, extracted from the data was "trying to play an active role in delivery". Three major categories were linked to core categories were "management of effective interaction", "attempt to access for health care provider" and "seeking trust to healthcare provider".
Conclusion: The finding of this study, indicated that mothers gain action/interaction strategies for having active role in vaginal childbirth.
Key words: *Child birth, Mother's role, Grounded theory, Qualitative study.*

P-17
Child bearing in HIV serodiscordant couples (HIV positive man with HIV negative woman) in Shiraz 2015-2016

Alahyari S.
Shiraz University of Medical Sciences, Shiraz, Iran.
Email: dr.soheylaalahyari@gmail.com

Introduction: Most of the HIV positive persons are 20-40 years old and antiretroviral therapy increased their lifelong. The most important problem of these couples is havind children because of the risk of HIV transmission to woman and fetus with unsafe sex. Assisted Reproductive Techniques (ART) help to decrease or delete risk of HIV transmission.
Materials and Methods: In this study 3 HIV serodiscordant couples (HIV positive man with HIV negative woman) in Shiraz were evaluated and their infertility was ruled out, then sperm washing was performed to delete HIV which was confirmed with PCR, then IUI (intra uterine insemination) was performed for woman on ovulation time.
Results: The most cost benefit of ART for these couples is sperm washing and IUI, that performed for 3 couples (1 couple for 4 ovulation cycles and other couples for 2 ovulation cycles), these women became pregnant without HIV transmission.
Conclusion: Performing sperm washing and IUI in Shiraz HIV Center help for child bearing of many HIV serodiscordant couples in Iran without HIV transmission to woman and fetus.
Key words: *HIV, Child bearing, Serodiscordant couples.*

P-18
Study of related causes of discontinuance and continuance rate of pregnancy prevention methods among women referring to Urmia Shohada Health Center

Ghasemzadeh S, Ghareaghaji R, Kahorian O, Pouladi Y.
Students Research Committee, Urmia University of Medical Sciences, Urmia, Iran.
Email: esau.urmia@gmail.com

Introduction: From maturity to menopause, women are constantly worried about concerns associated with unwanted pregnancy. The only choices for them is abstinence from sex or use of pregnancy prevention methods. Prevention success depends on proper implementation of its methods. Stopping and changing the methods of prevention can lead to unwanted pregnancies, abortions and thus threatening the health of mothers and infants which could damage society.
Materials and Methods: This cross-sectional study was conducted between 2014-2015. Accordingly 134 person were sampling randomly. The data was collected via a questionnaire consisted of demographic and effective factors of changing pregnancy prevention methods, plannig pregnancy, unwanted pregnancy, and worrying about the ineffectiveness of the method. The data was analyzed by statistical software SPSS version 20 and using Coplan- Mayer statistic method, Chi-squared test, and variance analysis test. $P<0.05$ was considered statistically significant.
Results: Pregnancy prevention methods were most prevalent in the 15-24 yr old age group and the use of pregnancy prevention methods did not increase by the age and unwillingness to pregnancy. In this study, the use of OCP with 39.6% was the highest and the use of injection methodes with 4.3% was the lowest. The study showed that there is not a relationship between the level of educatio, awareness and unwanted pregnancy in women with discontinuance of pregnancy prevention methods. In this study there was a correlation between the number of pregnancies with discontinuance of pregnancy prevention methods ($p=0.009$) and women who dont want to be pregnant were more likely to

continue using of pregnancy prevention methods. (p=0.001)

Conclusion: Although family planning and contraceptives are widely available for families, according to the results, not only education programs regarding family planning before starting each pregnancy prevention method to women is recommended, but a complete incentive consultation about these methods is essential.

Key words: *Pregnancy prevention methods, Discontinuance, Unwanted pregnancy.*

P-19
Effect of ascorbic acid and menthone on the caspase 3 in the sperm cells of acyclovir treated rats

Dashti GhR[1], Toghiani S[2], Roudbari NH[2], Roozbehani S[3].

1. *Department of Anatomy and Molecula Biology, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
3. *Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.*

Email: dashti@med.mui.ac.ir

Introduction: Acyclovir (ACV) is a common drug used for treatment of Herpes virus infection. In this study, the role of this drug on apoptosis in rat spermatozoa was investigated along with the effectiveness of two herbal antioxidants of vitamin C (VC) and menthone in the prevention of ACV adverse effects was compared.

Materials and Methods: Albino Wister adult male rats with mean weight of 200-250 gr were divided randomly into six groups, each group with 6 rats.Treatment groups consisted of acyclovir alone (ACV) at the dose of 15 mg/kg/d, ACV (15 mg/kg/d) and VC at the dose of 20 mg/kg/d, ACV (15 mg/kg/d) and menthone at the dose of 100 µl/d, ACV (15 mg/kg/d) and menthone at the dose of 250 µl/d, ACV (15 mg/kg/d) and menthone at the dose of 400 µl/d and control group which ended the trial period without any treatment. The treatment period was 48 days. At the end of the experiment, twenty four hours after the last treatment, the animals were anesthetized and epididymal sperm cells from each rats were isolated in PBS to evaluate the expression of caspase3 enzyme by flow cytometry method.

Results: Results demonstrated a very strong supportive, protective effect along with impressive anti-apoptotic role of VC. The expression level of caspase 3 in VC receiving group was close to normal levels. Menthone, another antioxidant compound in the high dose level showed to have a strong anti-apoptotic power, against changes induced by ACV.

Conclusion: ACV adverse effects on the reproductive system were reversible by the VC and menthone which have significant ability of antioxidants and anti-inflammatory which may be promising in preventing apoptosis and enhancing spermatogenesis. The protective role of VC suggest a synergistic effect for antioxidant therapy.

Key words*: Caspase 3, Acyclovir, Vitamin C, Menthone.*

P-20
Protective effect of beta-carotene against titanium dioxide nanoparticles induced apoptosis in mouse testicular tissue

Daneshi E, Orazizadeh M, Khorsandi LS, Hashemitabar M, Absalan F.

1. *Anatomical Sciences Department, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
2. *Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

Email: erfan.daneshi@yahoo.com

Introduction: Many recent in vivo and in vitro studies have demonstrated that most nanoparticles (NPs) have an adverse or toxic action on male germ cells. NPs have the capacity to penetrate the blood-testis barrie. Among the various metal nanomaterials, titanium oxide nanoparticles (NTiO2) are used in a variety of consumer products such as sunscreens, cosmetics, clothing, electronics, paints and surface coatings. Recent studies demonstrated that NTiO2 induces apoptosis in various tissues including testis. Beta carotene (BC) possesses a wide array of pharmacological and biological activities including antioxidant, radioprotective, cardiovascular protection and anti-epilepsy. Therefore, this study was undertaken to evaluate the effect of BC on NTiO2-induced testicular germ cell apoptosis and expression of important apoptotic proteins including FasL, Bid, p38 MAPK and caspase-3 in mice.

Materials and Methods: The study groups consisted of 32 adult male NMRI mice. The mice were randomly divided into four groups, all of which contained 8 animals. The first (control) group received 0.2 ml normal saline for 35 days. The second group received 10 mg/ kg BC for 35 days. Third group (NTiO2-intoxicated mice) received 300 mg/kg NTiO2 for 35 days. Forth group (NTiO2+BC) initially received 10 mg/kg BC for 10 days and was followed by concomitant administration of 300 mg/ kg NTiO2 for 35 days. One day after the last administration, the mice were sacrificed by cervical dislocation under ether anaesthesia and testicles from each animal were dissected out and fixed in Bouin's solution. The samples were embedded in paraffin, sectioned (5 lm) for Immunohistochemistry studies and sectioned (3 lm) for TUNEL assay to detect apoptotic cells.

Results: In NTiO2-treated mice, expression of apoptotic related genes including Bid, FasL, caspase-3 and p38MAPK was significantly increased. Measurement of apoptosis using TUNEL and immunohistochemistry method showed significant increase in apoptotic index of germ cells in NTiO2-treated mice ($p<0.05$). TUNEL and immunohistochemistry assessments showed that the increase of apoptotic index of testicular germ cells in NTiO2-treated mice was reversed by BC.

Conclusion: Overall, our results provide new insights into the mechanisms involved in the process of apoptosis induced by NTiO2 in testicular tissue. Additionally, we showed that BC could effectively suppress the apoptotic pathways. BC has a potent protective effect against the testicular toxicity induced by NPs and might be clinically useful. Extrapolation of these data to the human situation is not appropriate. However, this information does provide a stimulus for true clinical investigations.

Key words: *Beta caroten, Ttitanium, Apoptosis.*

P-21

Pentoxifylline besides naltrexone recovers morphine-induced inflammation in male reproductive system of rats by regulating Tool-Like Receptor pathway

Shokri Asl V[1], Chehrei Sh[2], Jalili F[3], Jalili C[1], Ghanbari A[1].

1. *Department of Anatomy, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
2. *Department of Biology, Islamic Azad University Baranch of Arak, Arak, Iran.*
3. *Student Research Committee, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

Email: vahidshokriasl@gmail.com

Introduction: Morphine prolonged usage causes many harmful side effects on tissues. Pentoxifylline has many benefits on function of organs.

Materials and Methods: In present study, 40 rats were divided into 5 groups (n=8) and administrated with only saline, only morphine, pentoxifylline, pentoxifylline+ morphine, naltrexone+ morphine, respectively by the groups. Saline (0.09% NaCl), pentoxifylline (12 mg/kg) and naltrexone (10 mg/kg) were intraperitoneally injected daily, while morphine solphate (0.1, 0.2 and 0.3 mg/ml for 48 hours and 0.4 mg/ml from 7th day up to end) dissolved in water. The treatments were prolonged for 56 days for all groups. At the end of the experiments, the protein expression of TNF-α, IL-1β and Tool like Receptor-4 (TLR-4) in testis tissues were investigated by ELIZA method and the gene expression of TLR-2, TLR-3, TLR-4, TLR-5, TNF-α, IL-1β evaluated using real-time quantitative PCR. The data were assessed by One-Way ANOVA test followed by tukey's post hoc test using SPSS software for windows (version 20).

Results: The levels of inflammatory proteins showed a significant raised in both only morphine and naltrexone+ morphine groups in compared with other groups ($p \leq 0.001$) while these levels did not showed any significant changes in pentoxifylline, pentoxifylline+ morphine than saline group. The data of real time PCR showed that the ratio of gene expression in TNF-α, IL-1β and also TLRs (2, 3, 4 and 5) were significantly increased in only morphine and naltrexone+ morphine groups ($p \leq 0.001$) while there were no significant differences in pentoxifylline, pentoxifylline+ morphine in comparison with saline group.

Conclusion: Prolong usage of morphine can increase TNF-α, IL-1β and TLR-4 as inflammatory proteins levels and over expression of TLR-2, TLR-3, TLR-4, TLR-5, TNF-α, IL-1β genes in testis tissue. Pentoxifylline can reduce the inflammatory gene expression and protein levels of subsequent prolong usage of morphine.

Key words: *Morphine, Naltrexone, Pentoxifylline, Tool like Receptors, Rat.*

P-22

Interleukin-6 as a local regulatory factor on steroid secretion of human granulosa cells

Ghasemnejad T, Nouri M, Fattahi A, Latifi Z, Salmassi A.
Department of Reproductive Biology, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: tohidgasemnejad@gmail.com

Introduction: Numerous studies have shown that a variety of cytokines such as interleukin-6 (IL-6) can exert profound effects on ovarian function and probably also on reproductive processes such as gonadal steroid secretion, corpus luteum function, embryo development and implantation.

Materials and Methods: In control group, granulosa cells (GCs) were obtained from infertile patients undergoing in vitro fertilization and embryo transfer treatment and cultured for 72 hr. As the experimental group, they were given increasing concentrations of human recombinant IL-6 (8–128 pg/ml) in the absence or presence of FSH (96 U/ml).

Results: The results show that increasing amounts of IL-6 significantly inhibit E2 production in the absence or presence of FSH vs untreated controls (p=0.025 at IL-6=128 pg/ml and p=0.016 at IL-6=16 pg/ml respectively). IL-6 also inhibited FSH-stimulated but not unstimulated progesterone release (p=0.038 at IL-6=8 pg/ml).

Conclusion: These findings suggest that IL-6 may be one of the factors that plays a local inhibitory role on FSH stimulated E2 and progesterone release. It could be postulated that elevated local IL-6 levels may leads to endocrine reproductive dysfunction during genital infections.

Key words: *Interleukin-6, Granulosa cells, Progesterone, FSH, Estradiol.*

P-23

Effects of uncontrolled chronic restraint stress during pregnancy on the cerebral cortex and basal ganglia development in Wistar rat embryo

Rostamkhani F.
Department of Biology, College of Basic Sciences, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, Iran.
Email: shirinrostamkhani@yahoo.com

Introduction: Stress induction during the pregnancy may result in abnormal function of nervous system.
Materials and Methods: Female Wistar rats (W: 250g) were crossed with male rats and zero day of pregnancy was determined. Experimental group have been treated by restaint stress (45 min/day for 7 days). Seventeen days after the onset of pregnancy, the animals were anesthetized by ether and the embryos were taken out surgically. Weight of embryos were determined by a digital balance and lengths of Crown Rump (CR), Fronto Occipital (FO) and Bi Parietal (BP) as a criterion for embryos growth were determined by a Caliper. Then the embryos were fixed in Boin's solution and the tissues were processed, sagitally sectioned at 5-micrometer thickness and stained in H&E. The sections were investigated for cerebral cortex and basal ganglia development by light microscope and MOTIC software.
Results: Decrease of CR, FO and BP lengths and embryonic weight for the experimental group were significant. In Morphometric study, the thicknesses of frontal and parietal cortex in the embryos of experimental group were significantly reduced in camparison with control ones. In addition, the area of parietal and occipital cortex and basal ganglia were significantly decreased.
Conclusion: This study showed that maternal restraint stress will cause cerebral cortex and basal ganglia defects that certainly will lead to a lot of difficulties after birth.
Key words: *Stress, Cerebral cortex, Basal ganglia, Development, Wistar rat.*

P-24
Effects of amitriptyline and venlafaxine on sperm parameters, MDA and DPPH sperm of BALB/c mice

Bandegi L.
Department of Biology and Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: lbandegi@yahoo.com

Introduction: Prescribing antidepressant drugs is expanding. Considering the adverse effects of drugs on reproductive system and the increasing rate of infertility, evaluation of drug effects on infertility was necessary.
Materials and Methods: Forty adult male BALB/c mice were divided into five groups. Group1 (control) received distilled water, group 2 received amitriptyline (4 mg/kg), group 3 received amitriptyline (4 mg/kg) with vitamin C (10 mg/kg), group 4 received venlafaxine (2 mg/kg), and group 5 received vitamin C with venlafaxine (2 mg/kg) (10 mg/kg) to form the gavage for 35 days. After excision of caudal epididymis, the analysis of sperm parameters was performed. Also, for measurement of MDA and DPPH, sperm samples were collected.
Results: Sperm parameters in the group treated with amitriptyline decreased and in the group treated with venlafaxine increased and a significant difference was observed between the two groups in sperm parameters. The level of MDA test was higher in amitriptyline group compared to the venlafaxine group but it was not statistically significant. In DPPH test, no significant difference was observed between the groups.
Conclusion: Our findings indicated that the sperm parameters decreased significantly in the amitriptyline group compared to the venlafaxine group but to considering the decrease in the venlafaxine group compared to the amitriptyline group in MDA test, there was no significant difference between the two groups.
Key words: *Amitriptyline, Venlafaxine, MDA, Sperm parameters, Chromatin condensation.*

P-25
Evaluation of in vitro development of primordial follicle of encapsulated vitrified bovine ovarian tissue in alginate

Abdi Sh[1], Abdollahi Far M[2].
1. *Department of Anatomical Sciences, School of Medicine, Azad University of Medical Sciences, Tehran, Iran.*
2. *Department of Anatomical Sciences and Biology, Shahid Beheshti University of Medical Science, Tehran, Iran.*

Email: shabnam.abdi62@yahoo.com

Introduction: The ability to preserve small follicle and grow it to obtain matured oocyte in vitro would not only advance the understanding of germ cell development, but could significantly expand the number of oocyte available for assisted reproductive technologies. This will promote increased fertility in domestic animals, endangered species and young patient with cancer before radiotherapy and chemotherapy.
Materials and Methods: The ovaries from freshly killed heifers were collected and the outer cortex was removed in strips (up to 2 mm) using a scalpel. Ovarian fragments were equilibrated and vitrified. In second experiment, vitrified and non-vitrified ovarian tissue were capsulated with alginate hydrogel and cultured for 7, 14 days. At the end the growth and development of primordial follicle morphological assessment was done by hematoxylineosin staining and steroidogenesis and apoptosis was measured by TUNEL.
Results: The percentage of primary follicle and the level of hormone were significantly higher in capsulated vitrified and non-vitrified ovarian tissue in alginate. But the development of follicles in vitrified groups was lower than non-vitrified ovaries. The proportion of apoptotic cell was increased in all groups during the culture period, especially in vitrified sample.
Conclusion: The data suggest that encapsulated ovarian tissue in alginate could improve the development of primordial follicles in vitrified cultured ovaries.
Key words: *Alginate, Vitrification, Bovine ovarian tissue, Organ culture techniques.*

P-26
Evaluation of cryotop-vitrification of ovine oocytes on expression of PPARs genes (PPARα , PPARβ, PPARϒ) in early embryo development

Shefaee A, Kadivar A, Ahmadi E, BaratiF.

Department of Clinical Sciense, Shahrekord University, Shahrekord, Iran.
Email: shefaee70@yahoo.com

Introduction: Peroxisome proliferator-activated receptors (PPARs) are a member of nuclear hormone superfamily, which mainly regulate the expression of target genes involved in lipid and energy metabolism. These receptors are divided to three isotypes: PPARα, PPARγ and PPARβ (also known as PPARδ). Vitrification is an alternative method for cryopreservation in which cell are exposed to a higher concentration of cryoprotectants and frozen with an ultra-rapid freezing velocity; resulting in an ice crystal free, solid glass-like structure. In the present study we have investigated the effect of vitrification on embryos drived from vitrified ovine oocytes and fresh oocytes. The results shown that in vitrified oocytes compared with fresh, gene expression was decreased, but that is not significantly. Expression PPARβ was decreased in embryonic development, and aboute 3 genes vitrification cause of decrease of transcripts and this study indicant that vitrification has no significant effects on this 3 genes.
Materials and Methods: Ewe ovaries gathered from the local slaughterhouse and transported to the laboratory in at 30°C. The follicular content was aspirated in preincubated hepes-TCM and COCs vitrification was performed in accordance with the method of minimum essential volume using cryotops as device. Total RNA extraction was performed and after quality control by nanodrop, RNA was reverse transcribed into cDNA. The levels of all three PPAR transcripts were quantified by real time reverse transcriptase polymerase chain reaction. Beta-actin was selected as a housekeeping gene. The developmental ability of oocytes was compared by regression analysis in CATMOD procedure and mean percent of developmental ability in different stages was compared by t-test. Gene expression in different groups was compared by two way analysis of variance and means compared with Tukey test.
Results: The results showed that the mean percentage of viable oocytes, cleaved embryos on days 1 and 3, 6 days embryos and total embryos was significantly lower in vitrified group ($p<0.05$). The PPARβ gene expression showed a significant decrease in blastocysts compared to oocytes and this difference was observed in both vitrified and control group ($p<0.05$).
Conclusion: In conclusion, cryotop vitrification did not significantly alter the gene expression of PPARs family, but the expression of PPARβ showed significant decrease during the developmental stages.
Key words: *Peroxisome proliferator-activated receptors Gene expression, Vitrification*.

P-27
Evaluation of toxicity of two different carrier devices by quality control assays

Motamedzadeh L[1, 2], Hadinedoushan H[3], Farashahi Yazd E[1], Mohammadi MM[2, 4].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
2. *Department of Immunology and Allergy, Kerman University of Medical Sciences, Kerman, Iran.*
3. *Department of Immunology, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
4. *Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran.*

Email: lmotamedzadeh5858@gmail.com

Introduction: Quality control is widely used to assess toxicity and functionality of various disposed items in routine IVF procedures. Human sperm motility assay (HuSMA) and mouse embryo assay (MEA) are the most popular methods to ensure highest standard for gamete and embryo in assisted conception program.
Materials and Methods: Two carrier devices (Company A, Iran , Company B, Japan) were tested by HuSMA and MEA. HuSMA was conducted after 30 minutes and 1, 2, 4 and 24 hours after incubation intervals. Sperm motility index (SMI) was calculated. In MEA test, all mice embryos were selected randomly from a common pool of freshly collected zygotes. Percentage of fertilization was calculated 46 hours after HCG injection with formation of 2 cells embryos (Percentage of fertilization: Number of zygotes/ Number of 2 cell embryos). 2 cell mice embryos were vitrified and cultured in 37°C, 5% CO_2 incubator after thawing. Percentage of blastocysts formation was noted after 72 hr.
Results: The toxicity of two carrier devices (groups A and B) was not significantly different after 30 min, 1, 2, 4 and 24 hr ($p>0.05$). In MEA test, 80% of 2cell mice embryos in group A compared to 40% of 2cell mice embryos in group B developed to blastocyst after 72 hr ($p>0.05$). The percentage of blastocysts formation after 72 hr was 50.7% and 25.8% in groups A and B, respectively. So, two carrier devices were not confirmed regarding MEA test.
Conclusion: For selection of the most efficient items, the quality control programs must be performed before utilization of all disposable and materials routinely used in ART laboratory.
Key words: *Quality control, HAS, MEA, Embryo.*

P-28
Effect of oocyte vacuole size on fertilization, cleavage and embryo quality after intra cytoplasmic sperm injection

Fadavi Islam M.
Novin Infertility Treatment Center, Mashhad, Iran.
Email: mahlafadavi@gmail.com

Introduction: Cytoplasmic abnormalities especially those that interferer with meiotic spindle and cytoskeletal structure during oocyte maturation can adversely affect fertilization rate and embryo quality following intra-cytoplasmic sperm injection (ICSI). Oocyte vacuolization is a common seeing in ART Lab.

Researches in this field mention 3.9% of oocytes have vacuoles, of which 66% have single, 21.3% have double and 12.7% have multiple vacuoles. Using transmission electron microscopy, identify 2 different types of vacuole, smooth endoplasmic reticulum clusters (sERCs), formed by accumulation of smooth endoplasmic reticulum and 'fluid filled' vacuoles.

Materials and Methods: In this prospective study that performed in Novin Infertility Treatment Center 342 fluid filled vacuolated oocytes were evaluated. Vacuolated oocytes were divided in 3 groups based on vacuole size (A ≤10 μm, 10≤ B ≤14, C ≥14). The fertilization rate and embryos quality were compared between these groups. From 342 vacuolated MII oocytes, 135 (39.47%) were in group A, 114 (33.33%) were in group B and 93 (27.19%) were in group C. 420 normal morphology oocytes was considered as control group.

Results: There were no significant differences in fertilization rate (p=0.32) and embryo quality (p=0.13) between group A and control group. There was a highly significant difference in fertilization rate (p=0.01) and embryo quality (p=0.002) between group B and control group. From 93 MII oocytes with vacuole size ≥14, we had just 7 low quality embryos.

Conclusion: The data suggest that oocyte quality plays a major role in fertilization rate and embryo quality. Oocyte with macro vacuolization had failed to fertilize. It is the cause of infertility in some patients. It probably has a biological basis and possibly a genetic cause, resulting in either uncontrollable endocytosis or poor exocytosis and consequent vacuolar formation.

Key words: *Vacuole, Fertilization rate, Embryo quality, ICSI.*

P-29
Ultrastructure of cytoplasmic fragments in human cleavage stage embryos

Halvaei I[1], Khalili MA[1], Esfandiari N[2], Safari S[1], Talebi AR[1], Miglietta S[3], Nottola SA[3].

1. *Department of Clinical Embryology, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Division of Reproductive Endocrinology and Infertility, Department of Ob-Gyn, Geisel School of Medicine at Dartmouth, Lebanon, NH, USA.*
3. *Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, La Sapienza University of Rome, Rome, Italy.*

Email: ihalvaei@modares.ac.ir

Introduction: One of the most common methods in ART clinics is selection of embryos based on embryo morphology. Embryo fragmentation and debris in perivitelline space are the main dysmorphisms in the cleavage stage embryos.

Materials and Methods: One hundred and fifty intracytoplasmic sperm injection cycles with male factor infertility were included in this prospective study. Patients were divided into three groups of case (n= 50), sham (n= 50), and control (n= 50). Embryos with 10-50% fragmentation were included in this study. Cosmetic microsurgery and zona assisted hatching were only performed in case and sham groups respectively. Extracted fragments were evaluated ultrastructurally by transmission electron microscopy (TEM). Rates of clinical pregnancy, live birth, miscarriage, multiple pregnancies, and congenital anomaly in the three groups were also compared.

Results: Micrographs from TEM showed that mitochondria were the most abundant structures found in the fragments along with mitochondria-vesicle complexes, Golgi apparatus, primary lysosomes, and vacuoles. There were no significant differences in demographic characteristics, laboratory and clinical data, or embryo morphological features between the groups. The rate of clinical pregnancy in control, sham, and case groups had no significant differences (24, 18, and 18%, respectively). The rates of live birth, miscarriage, multiple pregnancy, and congenital anomaly were also similar between the different groups.

Conclusion: Our data demonstrated that cosmetic microsurgery on preimplantation embryos had no beneficial effect on ARToutcomes in unselected groups of patients. As mitochondria are the most abundant organelles found in cytoplasmic fragments, fragment removal should be performed with more caution in embryos with moderate fragmentation.

Key words: *Cosmetic microsurgery, Embryo fragmentation, Transmission electron microscopy, Mitochondria.*

P-30
Effecst of tamoxifen on parameters -chromatin of mice sperm

Sadeghi S[1], Talebi AR[1, 2].

1. *Department of Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reprodctive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: sepidesadeghi0@gmail.com

Introduction: tamoxifen is a selective anti-oestrogen receptor modulators commonly used for the treatment performance for idiopathic infertility. This drug is used with 20 mg/kg/daily for 3 or 6 month in azoospermia patients to increase in number of sperm for improve fertility. The aim of the present study was to evaluate the effects of used oral tamoxifen treatment on epididymal sperm chromatin compaction and nuclear chromatin condensation during spermiogenesis.

Materials and Methods: Totally 24 adult male Nmri mice (8 weeks old. 30g) were randomly divided into 3 groups (2 experimentals and one control) each containing 8 mice. Group 1 received basal diet and tamoxifen (0.4 mg/kg/daily, feeding tube), group 2 received basal diet and tamoxifen (0.6 mg/kg/daily, feeding tube) and group 3 as control animal receive vehicle (administered water instead of dug) for 35 days. Finally, right tail of epididymis of each mouse was cut and placed in Ham's F10 medium for 30 min. Released

sperm were used to analyze count (number) and viability. Sperm chromatin condensation was assessed by two different tests including Aniline blue (AB).
Results: Following tamoxifen administration on sperm counts and viability, no change was evident between the epididymal sperm counts of control and tamoxifen treated groups. Tamoxifen treatment led to a significant reduction on sperm chromatin condensation.
Conclusion: The results showed that tamoxifen administration has no detrimental effects on sperm count and viability, but have effects on chromatin quality in mice as an experimental model.
Key words: *Adtamoxifen, Sperm, Chromatin, Mice.*

P-31
Toll like receptors have a crucial role in male reproductive health

Saeidi S[1, 2], Shapouri F[2], Hoseinifar H[3], Sabbaghian M[3], Pacey A[4], Aflatoonian R[1].
1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, Australia.*
3. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK.*

Email: R.Aflatoonian@gmail.com

Introduction: Reproductive health is an important feature of the human individual and population growth. It is well known that infections can have inhibitory effects on male reproductive function by adversely affecting sperm function. So, interactions between the immunity system and reproductive tract have important consequences for fertility and reproductive health. Although numerous studies showed a trade-off between the immune system and reproduction, there is a little knowledge about the role of innate immunity system role in the protection of the male reproductive system. Toll-like receptors (TLRs) are one of the major components of the innate immune system and in many cell types, they play a critical role in the front line of host defenses against microbes. Up to now, 10 TLR family members have been identified in the human. Each of the TLRs is known to detect ligands from varying classes of microbial agents.
Materials and Methods: Considering the emerging importance of TLRs in innate immunity and the cooperation between reproductive and immune systems, we studied the presence and distribution of TLRs in different parts of human male reproductive tract (testis, epididymis, vas deferens, prostate and prepuce) and ejaculated human spermatozoa.
Results: The results of this study have shown that TLRs are present in all tissues of the male reproductive tract as well as ejaculated spermatozoa. Thus, probably there is a role for TLRs in all segments of the male reproductive tract.
Conclusion: As It has been suggested that malfunction of immune system may causes testicular cancer, in continue we decided to examine expression of TLRs 2, 3, 4 and 9 as main components of innate immunity in the human testicular cancer. The results of this study reveal that cancer samples had stronger expression of these genes compared with normal ones. It seems that the different TLRs expression in testicular cancer cells may contribute to extensive signaling pathways involved in carcinogenesis.
Key words: *Toll like receptor, Innate immune system, Male reproductive tract.*

P-32
Pregnancy outcome in women with thin endometrium undergoing frozen-thawed cycles after intrauterine infusion of autologous platelet- rich plasma (PRP)

Neghab N[1], Eftekhar M[1], Naghshineh E[2], Khani P[1].
1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Obstetrics and Gynecology, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: nosrat20@yahoo.com

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. Recently, intrauterine infusion of platelet-rich plasma (PRP) is described to promote endometrial growth and receptivity. PRP is prepared from fresh whole blood that contained several growth factors and cytokines including fibroblast growth factor (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VGEF), transforming growth factor (TGF), insulin-like growth factor I, II (IGF-I, II), connective tissue growth factor (CTGF) and interleukin 8 (IL-8).
Materials and Methods: This is a randomized clinical trial. Among 33 infertile patients with thin endometrium (below 7 mm) at the 12^{th}-13^{th} cycle day the patients received PRP to improve endometrial thickness by slow intrauterine infusion using IUI catheter and estradiol was increased to 10 mg. If the endometrium had not reached at least 7-mm within 48 hr then sec infusion was given. Endometrial thickness was assessed by serial vaginal ultrasound at the most expanded area of the endometrial stripe.Thirty three thin endometrium women as control group only received 2 mg three times a day estradiol.
Results: chemical, clinical and ongoing pregnancy were higher in PRP group (42.4% vs. 24.2%, 39.4% vs. 18.2%, 33.3% vs. 18.2%) but these results were not significant (p=0.191, 0.102, 0.260 respectively).

Conclusion: PRP may be effective in improving endometrium, and possibly pregnancy rates in frozen-thawed embryo transfer cycles.

Key words: *Platelet-rich plasma, Frozen thawed embryo transfer, Pregnancy rate.*

P-33
Myths, infertility and sexuality

Bokaei M[1], Simbar M[2], Yassini Ardekani SM[3], Alavi Majad H[4].

1. *Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3. *Department of Psychiatry, Research Center of Addiction and Behavioral Scinces, Shahhid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Biostatistics, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: mah_bokaie@yahoo.com

Introduction: Infertility is a multipart and often misunderstood situation, which is why there's so much misunderstanding surrounding it. Discovering the myths in the field of sexual health and also determining the misconceptions would describe the educational needs for providing sexual and reproductive health programs for infertile couples. This study was done to determine women's myths about infertility and sexuality.

Materials and Methods: The present study was a qualitative conventional content analysis study (part of PhD theses) conducted on 8 key informants and 15 infertile women until reaching data saturation. For rigor of the study, Guba and Lincoln criteria were used.

Results: Data analysis showed three classes of myths in sexual health and infertility. 1) Cultural, religious, or ethnic beliefs Age of women is not important to become pregnant Women are guilty in any type of infertility. 2) sex behavior and infertility Masturbation is one of cause of infertility The effect of the number of intercourses on fertility Importance of time table intercourse. 3) effect of diet on infertility The effect of eating warm-natured foods on the success rate of ART.

Conclusion: Sexual health education is an important part of infertility approach. It seems that cultural and behavioral consulting is the most effective on reducing sexual myths of infertile Iranian women.

Key words: *Misconceptions, Infertility, Qualitative study.*

P-34
The evaluation of individual human sperm quality after cryopreservation by cryotop and warming in 37 and 42°C

Tahmasebi M.

Department of Embryology, Tarbiat Modares University, Tehran, Iran.

Email: moloud.tahmasebi@modares.ac.ir

Introduction: Nowadays, cryopreservation of individual sperm is a new way for male fertility preservation with low sperm concentration in seminal plasma and testis. Finding the best method for single sperm cryopreservation and warming with a suitable temperature can improve the sperm quality after recovery in such cases.

Materials and Methods: After swim-up of collected semen samples (16 normospermic cases), progressive motile sperm was selected by microinjection microscope and vitrified on cryotop with two mediums 1) human tubal fluid (HTF) with human serum albumin (HSA), sucrose (0.2 mM), taurine (50 mM) and 2) HTF with HSA and cryoprotectant agent (CPA). After three days, each group was warmed in 37 and 42°C. Finally the recovery rate , motility and viability was recorded.

Results: The sperm recovery rate did not show any significant difference among four studied groups [CPA 37°C: 98.1%, CPA 42°C: 98.1%, CPA free 37°C: 99.7%, CPA free 42°C: 97.4%]. The motility rate increased in 42℃ in both vitrified CPA and CPA free groups in comparison 37°C but it was only significant for CPA group [CPA: 80.0% vs. 62.5% ($p<0.05$)]. Moreover, the percentage of sperm motility in CPA group was significantly higher than CPA free group in both 37 and 42°C warming temperatures [62.5% vs. 38.7% and 80.0% vs. 50%, respectively ($p\leq0.05$)]. The survival rate of CPA group in both temperatures showed significant better rate in contrast to CPA free group [37°C: 73.1% vs. 54.7%; 42°C: 83.8% vs. 64.0% ($p\leq0.05$)].

Conclusion: Cryotop is a safe and effective tool for vitrification of limited sperms with at least missing sperm. In addition, it seems using vitrification with CPA and warming with 42 can improve sperm motility and viability rate during this procedure.

Key words: *Human sperm, Vitrification, Cryotop, Warming temparture, CPA.*

P-35
Correlation between sperm parameters and sperm DNA fragmentation in fertile and infertile men

Sheikh N, Amiri I.

Department of Biochemistry and Nutrition, School of Medicine, Molecular Medicine Research Center, Hamadan University of Medical Sciences and Health Services, Hamadan, Iran.

Email: amiri44@yahoo.com

Introduction: It is established that sperm DNA integrity is essential in fertilization and normal embryo and fetal development. Routine semen analysis gives an approximate evaluation of the functional competence of spermatozoa, but does not always reflect the quality of sperm DNA. Therefore, the evaluation of sperm DNA integrity, in addition to routine sperm parameters, could add further information on the quality of spermatozoa and reproductive potential of males.

Materials and Methods: Semen samples were collected from 45 infertile men selected from couples attending the infertility clinic with a history of infertility of ≥1 yr and 75 healthy volunteers of proven fertility (initiated a successful pregnancy) served as the control group. After routine sperm analysis, DNA damage was determined using single cell gel electrophoresis (comet) assay method.
Results: The mean of DNA damage (comet value) in the sperms of infertile males was significantly higher than that of fertile males (12.9±7.59 vs. 48.77±24.42, $p \leq 0.001$). A significant negative correlation was observed between DNA damage and sperm motility in fertile group ($p \leq 0.02$, R=-0.263). In infertile males, significant negative correlations were observed between DNA damage with sperm motility ($p \leq 0.002$, R=-0.45) and morphology ($p \leq 0.03$, R=-0.317). There was no significant correlation between sperm concentration and sperm DNA damage in both groups.
Conclusion: These results indicate that sperm DNA damages in infertile males is significantly higher than fertile males and sperms with abnormal morphology and low levels of motility has more abnormal DNA damages than motile and normal sperms.
Key words: *Sperm parameters, Male infertility, DNA integrity, Comet assay.*

P-36
NOX5 expression in healthy vs. oligozoospermic men before and after the treatment with palm pollen: a clinical trial

Izadi Raeini M[1], Amini A[1], Allameh Zadeh Z[1], Fallahi S[2], Malekzadeh K[1].
1. *Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
2. *Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*

Email: maliheizadi@yahoo.com

Introduction: Exclusive cause of about 20% of infertilities is the male factor. In the 20-40% of the rest, this factor still plays a role. Oxidative stress is considered an effective factor in male infertility. There is abundant evidence that palm pollen (DPP) possesses antioxidant properties which improve sperm quality. A body of research indicated that in many cells, physiological ROS values are produced by enzymes of NADPH family (NOX). If the spermatogenesis is disrupted and excessive cytoplasm is retained, ROS gets increased in abnormal sperm.
Materials and Methods: In this research, 40 oligozoospermic men and 10 healthy men participated. The infertile group was treated for one month with gelatin capsules containing DPP. Semen samples were obtained from all subjects both before and after the treatment. To assess sperm parameters, free 8-Isoprostane was tested as an index of DNA damage. mRNA expression of *NOX5* gene was tested in two phases.
Results: Expression of NOX5 was higher in the oligozoospermic group than the control. Infertile men treated with DPP had a significant reduction in the expression of mRNA of NOX5 ($p<0.05$). A similar reduction was also observed in free 8-Isoprostane and sperm parameters were improved ($p<0.05$).
Conclusion: These findings revealed that oxidative stress was truly a key factor involved in male infertility. DPP showed a positive effect on fertility and reducing sperm oxidative stress. This herb can, therefore, be used as a low-risk therapeutic approach in treating male infertility.
Key words: *Infertility, Oligozoospermia, DPP, NOX5, ROS.*

P-37
Vitrification of low number of human spermatozoa with close system preserves cellular and molecular integrity better than open system

Hosseini A, Khalili MA, Talebi AR, Sheikhha MH, Ghasemi-Esmailabad S, Fesahat F.
Reseasrch and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: hosseinia30@yahoo.com

Introduction: Nowadays, cryopreservation become the routine technique that is used in ART centers for long term storage of the human spermatozoa. Despite the reasonable success, the freezing-thawing process is associated with alterations of sperm quality.
Materials and Methods: Semen samples from 30 normozospermic men were prepared according to the routine swim-up procedure and divided into 4 aliquots for comparison of fresh and vitrified spermatozoa from the same ejaculate. After loading spermatozoa on the straws, in open system, straws were immersed into LN2. In one of the close system, straws were covered with plastic caps and then put in LN2, while in other close system, after recaping, straws were inserted into the other protective straws. The protective straws were then sealed before plunging into LN2 in order to samples not to be exposed to LN2. Conventional sperm parameters such as motility, DNA and chromatin integrity and a morphological analysis at high magnification (×6000) using image analysis software were performed before freezing and after thawing. The level of expression of Heat-shock protein (HSP) 70, 90, A2 and HO-1 were analyzed according to the expression level of the housekeeping β-actin gene.
Results: Vitrification induced some alterations in organelle morphology of motile human spermatozoa and altered the proportion of sperm motility, chromatin and DNA integrity when compared to control. RT- PCR analysis showed the levels of gene expression were affected by freezing-thawing process.
Conclusion: Vitrification process could affect the sperm cellular and molecular integrity, and these alterations were worse in vitrification with open system.
Key words: *Sperm vitrification, Open system, Close system, Expression gene.*

P-38
Effect of pentoxifylline on DNA fragmentation, MSOME morphology and fertility potential in frozen-thawed sperm with vitrification

Yari N, Etebary S, Khalili MA, Talebi AR.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: khalili59@hotmail.com

Introduction: Sperm cryopreservation is used in assistant reproductive technology (ART), gamete preservation before cancer treatment, and sperm banking. Cryopreservation affects sperm structure, in particular, in the head, the midpiece, and the tail regions. Most of the methods currently used to evaluate sublethal damage to spermatozoa are invasive. Motile sperm organelle morphology examination (MSOME) under high magnification (×6600) could allow evaluation of morphology and nuclear integrity of spermatozoa. Also, sperm motility is particularly sensitive to cryodamage. Several agents have been defined to increase sperm motility. Pentoxifylline is added to the semen in the laboratory, and stimulate motility of the spermatozoa. Although using this agent to achieve sperm motility has recently drawn attention, there is no consensus on whether this has toxic effect on the sperm cells. The present study aimed to evaluate the influence of PX on frozen-thaw human sperm motility, viability, DNA fragmentation, acrosome status, mitochondrial membrane potential and motile sperm organelle morphology examination (MSOME).
Materials and Methods: In this experiment, semen samples of 30 normozoospermic men (20-40 yr old) were studied. Each sample was swim-up prepared and divided into 3 equal parts. The first part was evaluated freshly as a control group (Group I) and the remaining two parts were used for vitrification whit open-pulled straws (Group II). PX was added to group III, after thawing. For all groups, sperm motility, viability, DNA fragmentation, acrosome status, mitochondrial membrane potential and fine morphology using MSOME were assessed.
Results: A significant reduction in class 1 Cassuto scoring spermatozoa was demonstrated after thawing in both groups of vitrification. There was positive correlation between class1 and high quality embryo. We observed significant changes in the viability, DNA fragmentation, acrosome status and mitochondrial membrane potential ($p<0.001$) in all groups, except acrosome status, which showed no significant differences ($p=0.231$). Vitrification increased the percentage of small vacuoles in spermatozoa, but not statistically significant. Sperm DNA and mitochondrial damage was significantly increased when samples are treated with pentoxifylline.
Conclusion: Toxicity, dose and duration of exposure to pentoxifylline should be evaluated in detail prior to application of this agent. Motile sperm organelle morphology examination (MSOME) is a technique that allows a more accurate selection of normal sperm. The present study proposes increase chances of success in ART cycles based on high magnification.
Key words: *Cryopreservation, Human spermatozoa, MSOME, Sperm motility, Mitochondrial membrane potential, Pentoxifylline.*

P-39
Effect of Palm pollen in infertile men: A before-after study

Fallahi S[1], Malekzadeh K[2], Rajaei M[3], Kalantar SM[4].
1. *Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
2. *Department of Molecular Medicine, Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
3. *Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
4. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Iran.*

Email: fallahi.leila@gmail.com

Introduction: A variety of factors explain male infertility ranging from hypothalamus-pituitary disorders to primary gonadal disorders, sex hormone disorders or sperm transmission disorders. Palm pollen is an herb contributing to the quality of factors related to fertility including hormones.
Materials and Methods: As a clinical trial, the present study has all men complaining about infertility as its population, who visited the single urology/infertility clinic in Bandar Abbas in 2015. The inclusion criteria were a sperm count below 20 millions per milliliter of seminal fluid and the age range of 20-40 yr. The exclusion criteria were affliction with certain infections and genetic diseases. The final sample size was set to be 44. Upon entering the experiment, all subjects had a blood sample test. For a month, they were treated daily with 4 gr of palm pollen. Near the end of the experiment, the test was administered again. The data entered SPSS ver.17 and were reported as mean, standard deviation and percentage. The statistical procedures were Wilcoxon's test as well as Spearman's correlation coefficient test($p\leq0.05$).
Results: The data belonging to 44 male subjects were statistically analyzed. The mean scores before and after the intervention were compared together and showed a statistically significant divergence ($p<0.05$). The mean BMI (kg/m^2) of all subjects was 32.43±5.76. Spearman's correlation coefficient of BMI and altered testosterone level was positive and moderate (0.291). Statistically speaking, it was close the significance level (0.055). Alteration in hormones before and after the treatment with palm pollen reached the significance level in all hormones ($p<0.05$). The only insignificant level belonged to estradiol level ($p>0.05$).
Conclusion: The present findings revealed that a daily consumption of 400 mg/kg of palm pollen for a month can raise the concentration of testosterone and FSH-LH hormones and lower the concentration of progesterone,

estradiol and prolactin. It also seems that consuming palm pollen can help to balance androgen and estrogen.
Key words: *Infertile men, Palm pollen, Hormons.*

P-40
Evaluation of sperm protamine deficiency and apoptosis in infertile men with morphologically abnormal sperm head

Dehghanpour F, Tabibnejad N, Fesahat F, Yazdinejad F, Talebi AR.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: dehghanpour64@yahoo.com

Introduction: Sperm morphology plays an important role in infertility especially in case of defects in head of spermatozoa. Tapered head or elongated head spermatozoa is kind of morphological abnormalities.
Materials and Methods: Fifty two semen samples (27 patients with tapered sperm and 25 fertile men) were collected and semen analysis was performed according to WHO criteria for each sample. Protamine deficiency and rate of apoptotic spermatozoa were evaluated using chromatin A3 (CMA3) staining and TUNEL assay respectively.
Results: Sperm concentration, motility and normal morphology in tapered head spermatozoa were significantly decreased compared with controls .The rate of CMA3-reacted spermatozoa (CMA3+) in case group was higher than controls. The rate of apoptotic spermatozoa (TUNEL positive) were significantly increased in case group with respect to the controls.
Conclusion: The results showed that the tapered head spermatozoa contain abnormal chromatin packaging and high rate of apoptosis which can be considered as an important reason of lower fertility potential in these patients.
Key words: *Sperm morphology, Tapered head, Infertility, Protamine deficiency, Apoptosis.*

P-41
The effects of vasectomy on epididymal morphology and sperm parameters in adult male Balb/c mice

Amini Mahabadi J.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: j.mahabadi64@gmail.com

Introduction: Vasectomy is a common method for birth control in men that causes several implications as well. However, new surgical techniques could decrease these implications significantly. Conditions such as vasectomy and likely the reversal procedures including vasovasostomy and vasoepididymostomy can have key effects on epididymal cell functions.
Materials and Methods: Twenty adult (age: 8 wk) male Balb/c mice, weighting 20-30 gr were used in the experiments. They were divided into 2 groups (vasectomy and sham). The operations were performed under sodium pentobarbital (40 mg/kg body weight, IP) anesthesia via a lower mid-abdominal incision. The left epididymis caput was fixed for histological studies and the right one was used for sperm count and motility.
Results: Progressive fast and slow sperm motility were significantly decreased in the vasectomized compared to the sham operation group ($p \leq 0.05$) and the number of immotile sperm in the vasectomized group was increased in comparison to control group. Sperm granuloma was seen in 60% of epididymis after vasectomy. Also, Histological study showed an increase in tubular lumen diameter, interstitial space and infiltration of immune cells in interstitial tissue in vasectomized group.
Conclusion: Vasectomy increases histopathological changes in epididymis and decreases the motility of sperm developing a reduction in fertility rates.
Key words: *Vasectomy, Epididymis, Mice, Sperm motility.*

P-42
Animal enriched serum for human sperm cryopreservation; Findings for serum containing fish oil and vitamin E

Seifi S[1, 2], Alizadeh AR[2], Shahverdi AH[2], Esmaeili V[2], Sharafi M[3], Habibi M[2], Maroufizadeh S[4], Sharbatoghli M[2].
1. *Department of Biology, Faculty of Sciences, University of Science and Culture, Tehran, Iran.*
2. *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Animal Science, Tarbiat Modares University, Tehran, Iran.*
4. *Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: soroush.seifi1988@gmail.com; shahverdi@royaninstitute.org

Introduction: Cryo-injuries in human sperm cryopreservation encouraged researcher to design a suitable protocol for sperm freezing. Supplementation of freezing media with an enriched serum obtained from animal fed a diet supplemented with fish oil (FO) and vitamin E (VITE) could be a suitable strategy to preserve the quality of sperm after cryopreservation.
Materials and Methods: To produce enriched serum, 16 rams were divided into four groups and fed diets according to following dietary groups: Control (CTR), vitamin E (VITE; 200 IU/ram/day), fish oil (FO; 40 g/ram/day) and fish oil +vitamin E (FO+VITE). Afterward, sperm samples were collected from 3 healthy men (totally 15 samples during five weeks). Samples were divided into sixteen equal experimental groups for cryopreservation in freezing media (SpermFreezTM, Fertipro) containing different concentrations (2.5, 5 and 10%) of animal enriched serum as follows: CTR, VITE, FO and VITE+FO. Moreover, FBS (2.5, 5 and 10%) and control medium (SpermFreezTM with no additives) were used as control groups.

Results: The highest significantly percentage of viability of frozen-thawed sperm (61±3.24%) was obtained in freezing medium containing 2.5% FO+VITE serum ($p<0.05$). Supplementation of medium with 10% in both enriched serum and FBS dramatically reduced the viability of post-thawed sperm ($p<0.05$). Aside from serum concentrations, VITE as well as FO+VITE groups significantly ($p<0.05$) improved the recovery rate of thawed sperm rather than FO, CTR and FBS.
Conclusion: It seems that low concentration of enriched serum contained FO+VITE can improved human sperm viability after freezing-thawing. More investigations for roles of omega-3 fatty acids and their metabolites along with antioxidants need to be considered for protection of sperm against cryo-injuries in freezing media.
Key words: *Enriched serum, Viability, Freezing, Sperm.*

P-43
Three-dimensional (3D) stereological methods apply for estimating the sperm head volume and sperm midpiece and tail length

Abdollahifar MA, Dadras S, Panahi S, Bayat M, Abdi Sh.
Department of Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: m_amin58@yahoo.com

Introduction: Evaluation of sperm morphology has been considered as one of the most important factors for a successful fertilization and determining sperm quality. Although the World Health Organization (WHO) recommend to evaluate various other constituent part of sperm morphology but little attention has been paid to the parameters of the volume of sperm's head and length of sperm's midpiece and sperm's tail, in spite of this fact that they play a part into sperm motility. Thus, studies have been limited to special regions; including sperm structure and three-dimensional (3D) stereological methods apply for estimating the sperm length. Stereological methods considered in this study because unbiased stereological techniques were used in this study are easy, fast and accurate.
Materials and Methods: The semen samples were analyzed. Seven sperm samples were selected from the normal subjects. Slides were made by applying a 10 µl drop of semen to a microscope slide and a smear air-dried. They were then stained with Diff Quik. The volume was estimated using the nucleator method. The sperm's head were sampled using an optical dissector. For each sampled nucleolus, two horizontal directions (intercept) were considered from the central point within the cell mambrane of sperm's head to the cell membrane of sperm's head. The sperm's midpiece and tail length were estimated by counting the number of intersections between the tails and Merz grid test line in an unbiased counting frame, superimposed on live images of sperms.
Results: The data showed that the sperm's head volume was on average 21.71 µm 3 length of sperm's midpiece and sperm's tail was on average respectively 15.12 µm and 33.46 µm.
Conclusion: The method presented here seems to be a useful and very efficient way to estimate the volume and lengths of randomly orientated sperms' head, sperm's midpiece and tails. The data supported that the sperm's head volume and sperm's midpiece and tails length can play important role in motility of sperm.
Key words: *Stereology, Sperm, Morphology.*

P-44
Effects of supplementing different concentrations of L-Carnitine in lecithin-based semen extender on ram semen quality after freeze thawing process in non-breeding seasons

Jafari S, Vaseghi Dodaran H, Daghigh Kia H.
Department of Animal Science, University of Tabriz, Tabriz, Iran.
Email: sepehrjafari8@gmail.com

Introduction: The cell membrane of spermatozoa contains high amounts of polyunsaturated fatty acids. L-Carnitine is necessary to shuttle fatty acids across the sperm mitochondrial membrane. The mitochondria is responsible for creating, sustaining and managing sperm energy. L-carnitine has crucial role in the generation of metabolic energy.
Materials and Methods: Semen samples were collected from 5 healthy and mature ram using an artificial vagina. Different concentrations of 35, 70 and 105, mM L-Carnitine were diluted with lecithin-based semen extender and Cooled to 5°C and then placed in liquid nitrogen vapor and finally were stored in liquid nitrogen tank. The experiment was carried out on the basis of completely randomized design with 5 replicates.
Results: The results showed the highest total motility and progressive motility in extender containing 70 and 105 mM L-Carnitine that were significant compared to the control group ($p\leq0.05$). The lowest motility recorded in this experiment was the first level of L-Carnitine. WOB, VAP, MAD, ALH and VCL were significant compared to the control group. The first level of L-Carnitin was statistically significant in morphology than the control group. L-Carnitine in 105 mM improved membrane integrity compared to the control group ($p\leq0.05$). MDA was not significant at any level.
Conclusion: Supplementation of L-Carnitine in lecithin-based semen extender improve semen quality of ram and reduce negative effects of freeze-thawing process.
Key words: *Semen, L-Carnitine, Lecithin, Freezing-Thawing.*

P-45
Effects of low doses of exogenous thymoquinone on sperm motility and viability of normozoospermic men

Dashti GhR.
Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: dashti@med.mui.ac.ir

Introduction: Sperm motility is a highly complex molecular process which is the result of transverse waves exist along its flagellum. Thymoquinone (TQ) is the most abundant active component isolated from black seed (Nigella Sativa). It was revealed that in-vivo administration of thymoquinone could improve spermatogenesis and increase number and motility of spermatozoa.
Materials and Methods: Twenty semen samples of normozoospermic men were washed in modified Ham's F10 medium containing albumin. 10 semen samples were used for each concentration of thymoquinone (5 and 10 µg/ml). Each sample was washed and two aliquots of it were incubated with or without considered dose of thymoquinone. Sperm motility and viability were assessed after two hours of incubation. Also, sperm motility was graded as fast- and slow-progressive, non-progressive and immotile.
Results: Both doses of thymoquinone increased the percentage of total motile and fast-progressive sperms. Administration of 10 µg/ml of thymoquinone increased the percentage of slow-progressive sperms while the dose of 5 µg/ml reduced it. The percentage of non-progressive and immotile sperms was decreased but the percentage of viable sperms was not changed after using thymoquinone.
Conclusion: Low doses of thymoquinone can increase sperm motility in culture media.
Key words: *Nigella sativa, Spermatozoa, Sperm motility, Thymoquinone.*

P-46
The effect of Resveratrol on apoptosis and NLRP3 complex expression in experimental varicocele rat model

Hajipour E[1], Jalali Mashayekhi F[2], Mosayebi Gh[3], Baazm M[1].
1. *Department of Anatomy, Arak University of Medical Sciences, Arak, Iran.*
2. *Department of Biochemistry, Arak University of Medical Sciences, Arak, Iran.*
3. *Department of Immunology, Arak University of Medical Sciences, Arak, Iran.*

Email: elnazhajipour69@gmail.com

Introduction: Varicocele is one of the common cause of male infertility. Varicocele is an abnormal dilation in the testicular vein and caused hypoxia, reactive oxygen species accomulation, elevation in testicular tempreature, promote apoptosis and increase proinflammatory cytokine production. According to the varicocele pathophysiology; it is possible that a group of cytosolic receptors called NLRP3 inflammasomes also involve in varicocele pathogenesis.
Materials and Methods: In this study, 42 male Wistar rats were randomly divided into 7 groups (6 rats in each group): Control, left experimental varicocele (LEV), LEV +ethanol , LEV +20 mg/ kg resveratrol (RES), LEV +50 mg/kg RES, normal +20 mg/ kg RES, normal +50 mg/kg RES. Varicocele was induced by partial ligation of the left renal vein.Three months after varicocele induction, resveratrol was orally administered to rats for 1 month. The expression levels of NLRP3, ASC, Caspase-1, Bax and Bcl2 were analyzed using Real time PCR.
Results: Our results showed that resveratrol at both doses significantly ($p \leq 0.05$) decreased the gene expression levels of ASC, NLRP3, Caspase-1 and Bax and increased Bcl2 gene expression at high dose.
Conclusion: Resveratrol by reducing inflammatory factors and decreasing apoptosis can be used for adjuvint therapy to reduce varicocele complication.
Key words: *Apoptosis, Inflammasomes, Resveratrol, Varicocele.*

P-47
Does insulin replacement and Omega-3 protect the male reproductive function of streptozocine-induced diabetic mice?

Yaghoobi A.
Kerman University of Medical Science, Kerman, Iran.
Email: a.yaghoobi@kmu.ac.ir

Introduction: Diabetes mellitus (DM) might have adverse effects on the male reproductive system. Several experiments have shown n-3 fatty acids could improve male reproductive capacity. Present study was performed to examine the effects of omega3 on sperms and testicular parameters in diabetic mice.
Materials and Methods: NMRI adult male mice were randomly divided into intact and diabetic groups (n=8). Streptozotocin induced diabetic animals were divided into 4 group of: diabetic-saline (Dia-Sa), diabetic-insulin (Dia-Ins), diabetic-omega3 (Dia-omg3), and diabetic-insulin- omega-3 (Dia-ins-omg3). Following confirmation of diabetes by rise in serum glucose, different treatments including 3U/100 g insulin subcutaneously and 400 mg/kg Omega3 orally were applied where applicable according to the treatment groups. Thirty five days later sperm parameters including the number, motility, progression and normal morphology, testis diameters and structure including germinal epithelium thickness, seminiferous diameter and Leydig cell number, and testosterone level were evaluated.
Results: Thirty five days after the onset of the diabetes, sperm number, viability, fast motility, testes volume, and serum testosterone decreased insignificantly in the Dia-Sa group compared with the intact animals. Neither insulin replacement nor omega-3 administration could significantly improve the outcome.
Conclusion: We might conclude that short periods of diabetes could not significantly affect the male reproductive function and insulin replacement does not apply profound effect on male reproductive system. Also, omega-3 has little effect on sperm parameters and testis structure of diabetic mice.

Key words: *Diabetes mellitus, Male infertitlity, Insulin, Omega3, Mice.*

P-48
The correlatin between human sperm motility and PPARγ protein expression

Mousavi M[1, 2], Shahverdi AH[2], Alizadeh AR[2], Sayahpour FA[3], Esmaeili V[2], Sharbatoghli M[2], Maroufizadeh S[4].

1. *Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran.*
2. *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Stem cells and Developmental Biology at Cell science Research Center, Royan Institute for Stem cell Biology and Technology, ACECR, Tehran, Iran.*
4. *Department of Epidemiology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: shahverdi@royaninstitute.org

Introduction: Mammalian spermatozoa are only cells performing their function outside the male body and plenty of their mRNAs are translated to functional proteins after fertilization. Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-inducible nuclear receptor that has been implicated in energy homeostasis and it modulates lipid and glucose metabolism. Since the sperm metabolism and producing sufficient energy for sperm motility , has important role in male fertility, the main objective of this survey is check the correlation of human sperm movement parameters and PPARγ protein expression.

Materials and Methods: Ejaculated sperm have been collected from normozoospermia (n=40) and asthenozoospermia men (n=40) referred to Royan Institue. The semen parameters were measured by CASA. PPARγ protein expression determined by Flow cytometry. The anti-PPARγ antibody (Thermo scientific) was used as primery antibody.Adipose cell and RBC cell were used Respectively as positive and negative control. Flow cytometry data were analyzed by Flowing software and whole data were analyzed using the MIXED procedure of SPSS2 Program.

Results: PPARγ protein expression levels as determind by Flow cytometry in normozoospermia and asthenozoospermia men had a significant direct correlation with sperm motility and percentage of progressive cells. As well as there was a significant indirect correlation with the expression of these proteins and percentage of immotile sperms.

Conclusion: Because of the existence significant correlation between PPARγ protein and human sperm movement parameters, it seems that treatment the asthenozoospermia men with PPARγ agonists, May be effective in the treatment of these patients improved motility.

Key words: *PPARγ, Sperm, Flow cytometry, CASA, Sperm motility.*

P-49
Pentoxifylline besides naltrexone recovers morphine-induced inflammation in male reproductive system of rats

Chehrei Sh[1], Shokri Asl V[2], Jalili F[3], Jalili C[2], Ghanbari A[2].

1. *Department of Biology, Islamic Azad University Baranch of Arak, Arak, Iran.*
2. *Department of Anatomy, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
3. *Student Research Committee, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

Email: sh-chehreii@iau-arak.ac.ir

Introduction: Chronic usage of morphine induces many side effects on body organs and many drugs relief these hazardous impacts of morphine.

Materials and Methods: In present study, 40 rats were divided into 5 groups (n=8) and administrated with only saline, only morphine, pentoxifylline, pentoxifylline+ morphine, naltrexone+ morphine, respectively by the groups. Saline (0.09% NaCl), pentoxifylline (12 mg/kg) and naltrexone (10 mg/kg) were intraperitoneally injected daily, while morphine solphate (0.1, 0.2 and 0.3 mg/ml for 48 hours and 0.4 mg/ml from 7th day up to end) dissolved in water. The treatments were prolonged for 56 days for all groups. At the end of the experiments, the diameters of seminiferous tubules, the maturity of germ line epithelium, testicular weight and sperm parameters were evaluated. The data were calculated by One-Way ANOVA test followed by tukey's post hoc test using SPSS software for windows (version 20).

Results: Testicular weight was significantly reduced in morphine group in compared to other groups ($p \leq 0.001$). The sperm parameters in pentoxifylline, pentoxifylline+ morphine did not show any significant differences in comparison with only saline group, whilst in only morphine and naltrexone+ morphine groups it was reduced ($p \leq 0.001$). The diameter of the seminiferous tubules and the maturity of spermatogonia in both only morphine and naltrexone+ morphine were declined significantly in compared to other groups ($p \leq 0.001$).

Conclusion: Morphine consumption induces side effects on male reproductive system outbreaking by decreasing the weight of testis, sperm parameters, the diameter of the seminiferous tubules and the maturity of spermatogonia in rats. Pentoxifylline besides naltrexone can claim these hazardous effects of morphine and this impact of pentoxifylline approve that recovery of dangerous effect of morphine in male reproductive system is a not dependent to classical morphine receptors.

Key words: *Morphine, Naltrexone, Pentoxifylline, Spermatogenesis, Rat.*

P-50
Effect of vitamin D on motility and apoptosis in asthenozoospermia men

Hosseini Gh, Taherimoghadam M.
Department of Anatomical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Email: ahoo_8267@yahoo.com

Introduction: Vitamin D has important role for maintaining calcium, phosphorus homeostasis and promoting bone mineralization. Recently, localization of vitamin D receptor in the human sperm was detected. However, the action of this vitamin in human male reproduction has not yet been clarified. In this study, we evaluated the effect of this vitamin on sperm motility and apoptosis in asthenozoospermia men.
Materials and Methods: The study was carried out on discharged semen samples of 7 infertile men who referred to IVF clinic of Imam Hospital in Ahvaz Jundishapur University of Medical Sciences. Samples were processed for swimming up. Supernatant was divided into control and experimental (received 20 µMol of vitamin D) groups. They were assessed for sperm motility by Makler chamber, apoptosis of sperm with Annexin-V and TUNEL according to the WHO guidelines.
Results: The results revealed that: 1- Apoptosis in sperm was significantly decrease (30.46% vs. 8.68%, p=0.008) 2- Progressive motility was significant increase (3% vs. 0%, p=0.004). 3- Total motile sperm was increased with vitamin D (9.21% vs. 3.88%) but there was no significant statistical difference (p=0.413). 4- For TUNEL assay, the analysis showed no statistical difference (90.77% vs. 91.49%, p=0.811).
Conclusion: In this study, apoptosis and progressive motility of sperm have improved after incubation with vitamin D so it may be used for therapeutic opportunities in sperm and male reproduction disorders.
Key words: *Asthenozoospermia, Vitamin D, Sperm motility, Apoptosis.*

P-51
Tissue regeneration by omentum: omental mesenchymal stem cells apply cartilage regeneration

Goudarzi Z.
Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: negaar.goudarzi@gmail.com

Introduction: It is well known that adult cartilage lacks the ability to repair itself. Mesenchymal stem cells are attractive candidates for cartilage repair due to their chondrogenic potential, ease of harvest, and ease of expansion in culture. Omentum tissue contains a population of rare progenitor cells capable of differentiating into bone, cartilage, muscle, and other connective tissues.
Materials and Methods: To culture cells by micromass method, after isolation of OMSCs, the number of cells per unit increased. OMSCs have been centrifuged and settled at the bottom of a tube. After confirmation of OMSCs via flow cytometry, they were co-cultured with cartilage extracts, and the chondrogenic differentiation was monitored. Furthermore, immunocytochemistry was performed for cartilage matrix protein collagen type-II (CT-II) production. Cartilage matrix-sulfated proteoglycans (PGs) production was determined via toluidine blue and alcian blue staining.
Results: Flow cytometry analysis for OMSCs revealed the positive expression of CD90, CD44 and the negative expression of CD45. Growth of cell mass shows that cells have secreted matrix and created chondroblastic tissue as well. Chondrogenic differentiation at presence of 50 µg/ml cartilage extract was proved by immunohistochemistry on day 21. Moreover, chondrogenic differentiation improved when levels of FBS serum in medium lowered. PGs production was assessed by alcian blue and toluidine blue staining.
Conclusion: These observations show that cartilage extract is able to induce differentiation of OMSCs into chondrocytes. Additionally, this study suggests that by combining OMSCs with an appropriate delivery vehicle, it may be possible to offer patients new therapeutic options.
Key words: *Mesenchymal stem cells, Omentum tissue, Differentiation, Chondrocytes, Cartilage extract, Micromass method.*

P-52
Efforts for generation of mouse embryonic stem cells on mouse embryonic fibroblast feeder layer in a medium supplemented with leukemia inhibitory factor in a microdrop culture system

Rezaee R[1], Sardari R[1], Khoradmehr A[1], Sadeghian-Nodoushan F[1], Avijegani N[1], Dehghani Ashkezari M[2], Aflatoonian B[1, 3, 4].
1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University Yazd, Iran.*
3. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: roshan_rezaee@yahoo.com; mehrarezoo@gmail.com

Introduction: The pluripotent mouse embryonic stem cells (mESCs) were initially derived from the inner cell mass of blastocysts with the potential for the future applications in animal studies in regenerative medicine.
Materials and Methods: BALB/c female mice were injected IP 10 IU of Pregnant Mare's Serum Gonadotropin (PMSG) was injected intrapritoneally (IP), followed by injection of 10 IU human chorionic gonadotropin (hCG), 48 h later. According to animal

ethics protocols, using a special flushing syringe under a stereomicroscope the oviducts were flushed to obtain embryos. The zona plucida of the blastocysts were removed by either Pronase or Thyroid Acid and then zona-free blastocysts were co-cultured in a microdrop of MEF feeder layer with a medium supplemented with LIF. After 3 days, the outgrowths were appeared and then mESC-like cells were mechanically passaged onto new feeders.

Results: Totally 32 blastocysts with different grades were obtained from two pregnant BALB/c mice. Day after co-culture on MEF in microdrop, blastocysts were attached, and 3 days later they formed round colonies with spherical and almost regular margin shape. Initially all 32 embryos were formed outgrowths, which 16 of them were passaged. Eventually, from 9 and 4 embryos mESC-like cells were remained in culture till passage 2 and 3 respectively and then colonies initiated differentiation.

Conclusion: Our data showed that mESC-like cells can be generated in a microdrop culture system, however, mESC lines has not established yet. Further works are in progress to establish mESC lines using this method.

Key words: *Mouse embryonic stem cells, Leukemia inhibitory factor, Microdrop culture system.*

P-53

Testicular organ culture supports homing of human spermatogonial stem cells after in vitro transplantation to azoospermia mouse testis model

Mohaqiq M[1], Movahedin M[1], Mazaheri Z[1], Amir Jannati N[2].

1. *Anatomical Sciences Department Tarbiat Modares University, Tehran, Iran.*
2. *Andrologia and Embryology Department, Avicenna Research Institute, Tehran, Iran.*

Email: mohaghegh@modares.ac.ir

Introduction: Spermatogonial stem cells (SSCs) are considered as the base of spermatogenesis. One of the most important advances in transplantation process of germ cells is inducing spermatogenesis with germ cells.

Materials and Methods: Human SSCs were obtained after 2-stage digestion in TESE sample of the patients who are under infertility treatment. The identification of these cells was confirmed by tracking PLZF and Oct-4 proteins. These cells are then labeled with Dil and have been transplanted in adult mouse testes that were treated by busulfan 40 mg/kg. Testes of the host were considered as the experimental group under the tissue culture conditions. Other group without transplant was considered as control group. After two weeks, testes of the host group were used for tissue processing, histological studies, Dil tracking and immunohistochemical studies.

Results: The results of histologic studies with Hematoxylin-Eosin staining showed that transplanted cells can be seen on the seminiferous tubule basement membrane but their presence in control group is very small or none. Most subsided cells responded positively to Dil tracking. Immunohistochemical studies in the experimental group proved that there was PLZF and Oct-4 proteins expression in cells subsided on the seminiferous tubule.

Conclusion: These results suggest that testicular tissue culture conditions after transplantation of SSCs can support subsiding of these cells on the seminiferous tubule basement membrane.

Key words: *Stem cell, Human, Transplant, Azoospermia, Tissue culture.*

P-54

Preparation of TESE derived cells conditioned medium as a niche for in vitro spermatogenesis

Akyash F[1, 2], Talebi AR[2], Yazdinejad F[2], Aflatoonian B[1, 3,4].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: b.aflatoonian@ssu.ac.ir

Introduction: In vivo spermatogenesis depends on growth factors secretion from various sources of testicular cells including, Sertoli cells, Leydig cells, spermatogonial stem cells (SSCs), spermatocytes, and other testicular somatic cells. Also, these growth factors mediated cell to cell interactions and play critical role in primordial germ cells (PGCs) differentiation into germ cells when these cells reside at the genital ridge. Therefore, conditioned medium (CM) which obtained from TESE derived cells could provide a niche to induce *in vitro* spermatogenesis. Preparation of TESE derived cells CM as a niche for in vitro spermatogenesis.

Materials and Methods: TESE samples that contained sperm from individuals with non-obstructive azoospermia disorder were used after fully-informed patient consent. After washing the TESE samples in DMEM /10% FBS medium, tissue fragments minced mechanically, following enzymatically treatment and were pelleted by centrifugation for 3 min at 200 gr. The supernatant was removed and pellet was seeded in the tissue culture flasks containing DMEM/20%FBS medium. Collection of the CM was performed 4 days after each passage, then filtered through 0.22-mm syringe filter and stored at -20^{o}C.

Results: Cells from TESE samples were successfully isolated, cultured, frozen and thawed. CM from TESE derived cells were collected 4 days after each passage and filter and stored at -20^{o}C for the future studies to induce *in vitro* spermatogenesis.

Conclusion: In sum, TESE derived CM which can be used as an *in vitro* niche which may contain growth factors to induce the specific signaling pathways that mediated male germ lineage development and subsequent gametes formation. Hence, in the future studies, we will use the TESE derived CM for generation of male germ cells from human embryonic stem cells (hESCs).

Key words: *Conditioned medium (CM), Human embryonic stem cells (hESCs), Male germ cells, Testicular sperm extraction (TESE).*

P-55
Preparation of cumulus cell conditioned medium as a potential niche for in vitro oogenesis

Tahajjodi SS[1], Khalili MA[2], Soleimani M[2, 3], Agha-Rahimi A[2], Aflatoonian B[1, 4, 5].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
4. *Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: s.tahajjodi@yahoo.com

Introduction: Cumulus cells are the oocyte nurse cells during oogenesis in vivo. They provide a suitable microenvironment contains growth factors and cellular interactions required for oogenesis. In vitro oogenesis also, can improve with such microenvironment. Proliferation and differentiation will increase under the influence of multiple growth signals in the presence of growth factors. Since defect of oogenesis is the cause of infertility in some infertile couples, in vitro generation of female germ cells can be an option in the future for regenerative medicine in female infertility disorders.

Materials and Methods: Cumulus cells were obtained from male factor infertile couples that were entered to ART cycle. After oocyte retrieval cumulus cells were separated from cumulus–oocyte complexes (COCs) with syringe needles carefully. Following washing cumulus cells were plated onto the central well dishes and cultured with DMEM supplemented with 10% FBS and 1µl/ml penstrep. The medium from cultures were collected every 3 days after subculture. The cumulus cell derived conditioned medium was filtered and stored at -20^{o}C.

Results: Cumulus cells were isolated and cultured onto a central well culture dishes. The proliferating cells were overspread from the attached cumulus clusters. An obvious proliferation was observed after 7-8 day after primary culture and cumulus cell clusters were dispersed over central well culture dish. Cumulus cell derived conditioned was collected and stored for the future studies in artificial oogenesis.

Conclusion: Cumulus cells can attach and proliferate in culture to produce a potential conditioned medium which may contain growth factors and other important signals to induce artificial oogenesis as an in vitro niche for female germ cell development. Further works are in progress to characterize these cells with specific gene expression profile.

Key words: *Cumulus cells, Conditioned medium, In vitro oogenesis, Niche.*

P-56
The anticancer effect of Aloe Vera extraction on BAX expression in AGS cells

Tariverdi N, Deylami Khiabani Z.

Department of Genetic, Zanjan Branch, Islamic Azad University, Zanjan, Iran.

Email: nazilatariverdi68@gmail.com

Introduction: Gastric cancer is a major cause of cancer death. In terms of incidence it is the fourth most common cancer and the second leading cause of cancer death in the world. Aloe Vera plant belongs to the Liliaceae family and contain a variety of valuable minerals, vitamins, amino acids and antioxidant.

Materials and Methods: The present study is case-control. The aqueous extract of Aloe Vera were prepared in different concentrations. AGS adenocarcinoma cells were treated with aloe Vera aqueous extract in different groups and times. RNA extraction and cDNA synthesis was performed, and gene expression of BAX was evaluated by Real time PCR. Finally the obtained results were analyzed by statistical software.

Results: Expression of BAX gene at 72 and 48 hr, showed significant increase only in 800 µg/ml dose.

Conclusion: Aloe Vera extract increases the expression of Bax in 48 hr and this change leading the gastric cancer cells to apoptosis. The results was showed significant difference at 800 µg/ml dose and can be effective in improving gastric cancer.

Key words: *Aloe Vera, Bax, Gastric cancer.*

P-57
Expression analysis of MMP-15 in women with preeclampsia using cell free fetal RNA in maternal plasma

Etesami E[1], Ghotbizadeh Vahdani F[2], Shahhoseini M[2], Favaedi R[3], Ghaheri A[2], Nikukar H[1, 5], Zamanian MR[2].

1. *Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Genetics, Royan Institute for Reproductive Biomedicine, Reproductive Biomedicine Research Center, ACECR, Tehran, Iran.*
3. *Department of Obstetrics and Gynecology, Vali-Asr Hospital, Tehran University of Medical Sciences, Tehran, Iran.*

4. *Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
5. *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: e_etesami@yahoo.com

Introduction: Preeclampsia (PE) is a systemic maternal syndrome characterized by the new onset of hypertension and proteinuria after 20 wk of gestation. This condition affects 5-10% of all pregnancies and is the leading cause for maternal and perinatal morbidity and mortality. The only effective treatment for preeclampsia is delivery of the placenta. Identifying biomarkers for early detection and monitoring at risk pregnant women is necessary in order to decrease feto-maternal morbidity. Nucleic acid fragments released from the placenta into maternal circulation is possibly providing an important source for such novel biomarkers. Previous studies suggested that altered placental expression of matrix metalloproteinases (MMPs) coincide with defective cytotrophoblastic invasion and incomplete remodeling of the spiral arteries which ultimately lead to preeclampsia. We evaluated expression of MMP-15 in preeclamptic women using cell free fetal RNA and compared it with normal pregnancies.
Materials and Methods: Peripheral blood samples were obtained from 20 women with PE (28-32 week) and 20 gestational age-matched healthy pregnant controls. cell free fetal RNA was extracted using the QIAamp Circulating Nucleic Acid Kit Followed by cDNA synthesis using VILO SuperScript kit. Expression of MMP-15 was determined through Real-time PCR.
Results: Our results showed that expression level of MMP-15 gene in preeclamptic women was significantly higher compared to control group.
Conclusion: Cell free fetal expression level of MMP-15 has the potential to be used as a PE biomarker in pregnant women.
Key words: *Preeclampsia, Cell free fetal RNA, MMP-15.*

P-58
Haplotype analysis of BRCA1 intragenic markers in Iranian patients with familial breast and ovarian cancer

Miresmaeili SM[1], Kordi-Tamandani DM[2], Kalantar SM[3], Moshtaghioun SM[4].

1. *Department of Biology, Science and Art University, Yazd, Iran.*
2. *Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Biology, Faculty of Sciences, University of Yazd, Yazd, Iran.*

Email: smm230@gmail.com

Introduction: Breast cancer is the most common malignancy in women. Breast Cancer Type 1 Susceptibility gene (BRCA1) is a tumor suppressor gene, involved in DNA damage repair and in 81% of the breast-ovarian cancer families were due to BRCA1. In some clinically investigated genes, the intragenic marker polymorphism is important and the screening of such mutations is faster by using short tandem repeat (STR) polymorphism. Individual polymorphism of STR is a good evidence for following inheritance of repeat polymorphism.
Materials and Methods: A total of 107 breast and/or ovarian cancer patients and 93 unrelated healthy women with no clinical phenotype of any malignancy or familial cancer history constitute the study groups. Haplotyping analysis, at 3 intragenic BRCA1 microsatellite markers (D17S855, D17S1322 and D17S1323), were performed for all subject and control groups using labeled primers.
Results: After fragment analysis, significance differences were observed as follows: two alleles of D17S855; allele 146 (p=0.02) and 150 (p=0.006), and two alleles of D17S1322, allele 121 (p=0.015) and 142 (p=0.043). These differences were compared with control group. There was significance difference in 8 di/tri allelic haplotypes in present experimental subjects. Some haplotypes were observed to have approximately twice the relation risk for breast cancer.
Conclusion: According to recent results, assessment of presence or absence of mentioned alleles in BRCA1 microsatellite can be used for prognosis in individuals, suspected of having or not having the breast cancer.
Key words: *Breast cancer, Ovarian cancer, STR haplotyping.*

P-59
Evaluation of methyl-beta-cyclodextrin and electroporation for Rooster sperm mediated gene transfer

Rahimi M[1], Rahimi Sh[1], Shahverdi AH[2], Sharafi M[1], Esmaeili V[2].

1. *Department of Poultry Science, Tarbiat Modares University, Tehran, Iran.*
2. *Department of Embryology, Royan Institute, Tehran, Iran.*

Email: mrahimi5988@gmail.com

Introduction: Sperm mediated gene transfer can be one of the best strategy to produce transgenic chicken. Transfected sperms should be alive and fertile to be executable in artificial insemination of flocks of hen. However, efficiency of gene transfection via sperm is not desirable in animal. Different chemicals and physical protocols such as liposomes and Electroporation have been used to improve this efficiency.
Materials and Methods: In order to transfection, hG-CSF gene were synthesized in PBHA vector and then cloned in PcDNA3.1+ expressing vector. Semen samples were collected from six roosters and were washed twice to prevent DNase activity.Then, PcDNA3.1+/ hG-CSF vector was transfected to the

sperm cell by using electroporation (group1) or Methyl-Beta-Cyclodextrin (group 2). Absorption of transgene by rooster sperm cells were investigated by using PCR reaction. Moreover, Total and progressive motility, velocity parameters (VCL, VSL, VAP, LIN and STR), membrane integrity and viability were checked after transfection.

Results: PCR reaction recognized the existence of hG-CSF gene in rooster sperm. Electroporation showed a significant decrease in the total motility (86.9±1), progressive motility (40.3±2.6), membrane integrity (70±2.1) and viability (69.9±1.5) compare to incubation with MCDB. Morever incubation with MBCD treatment produced higher significant of viability and membrane integrity (84±1.5, 83.02±2.1 respectively) compared to electroporation treatment (69.9±1.5, 70±2.1 respectively).

Conclusion: It is concluded that MBCD is more efficient for protection of sperm in sperm mediated gene transfer.

Key words: *Sperm mediated gene transfer, Motility, Velocity parameters.*

P-60
Evaluation of genetic variations in exon 7 of *SEPTIN12* Gene in infertile men with teratozoospermia

Rafaee A[1], Yaghmaei P[1], Mohseni Meybodi A[2], Sabbaghian M[3].

1. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: sadafrafaee@gmail.com

Introduction: Teratozoospermia is a condition characterized by the presence of spermatozoa with abnormal morphology in semen samples. *SEPTIN12* is a potential sterile gene in human which belongs to a family of polymerizing GTP-binding proteins and is critical for sperm normal development. SEPT12-microtubule complexes are required for sperm head and tail formation. Mutations and genetic variants of *SEPTIN12* have been observed in teratozoospermic men with distinctive sperm pathology including defective sperm tail elongation and sperm head abnormalities. In the current study we investigated exon 7 of *SEPTIN12* gene variants in infertile men with teratozoospermia.

Materials and Methods: In this study 30 infertile men with short tail sperm, 30 patients with round-headed sperm and 30 normozoospermic men as control group were recruited. To investigate genetic variations, DNA was extracted from peripheral blood samples using salting out method and mutational analysis was performed by direct sequencing of exon 7.

Results: No mutations or single-nucleotide polymorphisms (SNPs) were found in exon 7 of *SEPTIN12* gene in cases and control groups, however an intronic variant (rs: 7201715, T>C), was observed within intron 7-8 in homozygote and heterozygote forms in 2 and 1 patients with short tail sperm respectively. One of the patients with round-headed sperm had this variation heterozygously. This variation was also identified in 3 normozoospermic men heterozygously.

Conclusion: Although obtained data indicated no association between exon 7 of *SEPTIN12* gene variations and teratozoospermia, because of SEPTIN12 essential role in sperm head and tail development, evaluation of other exons of this gene is recommended. Moreover, the T>C intronic variation is located within the binding site of hnRNP K splicing factor in pre-mRNA. Thus bioinformatics analysis would reveal the possible impact of this variation on splicing process.

Key words: *Teratozoospermia, SEPTIN12, Genetic variation.*

P-61
Comparing the teratogenic effects of agricultural peseticides of (diazinon and chlorpyrifos) on embryos Balb/C mice

Rastgar Gharahshiran S, Torabzadeh P, Saffari S.

Department f Biology, Karaj Branch, Islamic Azad University, Karaj, Iran.

Email: safoora_saffari_66@yahoo.com

Introduction: Diazinon and chlorpyrifos are agricultural pesticides and organophosphate pesticide. Their destructive effects in recent years have been studied. But the effects these pesticides on the embryo have not been reviewed.

Materials and Methods: Was studied two groups of 50 mice and divided randomly each into 6 groups as control group (non-injection: 5mice) and sham (injection of saline: 5mice) and 4 experimental groups (each group: 10 mice). Lethal doses of LD50 diazinon and chlorpyrifos were determined in condition of 11.09 and 2./32 ml/kg.bw in vivo and selected dose for injection was 0.4 ml/kg.bw. Injection was done on the 3rd to 6th days of pregnancy by enema Then mice were sacrificed on day 15 of pregnancy. Data was checked with SPSS17 software and Duncan test subjest to ($p\leq0.05$) and ($p\leq0.001$).

Results: The results showed that teratogenic effects of diazinon and chlorpyrifos was more intense, as the experimental groups observed a significant increse in abnormalities such as exohepatic, exencephalus, syndactyly, having a defect in dynamic organs (legs and hands), extensive bleeding in whole body and exophthalmia, compared with control and sham groups.

Conclusion: According to the findings of this study, the use of agricultural pesticides diazinon and chlorpyrifos, have negative effects on the mouse embryos and are teratogen. It is recommended to protect the environment

and human health, especially pregnant women by using non-chemical methods to control pests.

Key words: *Agricultural pesticides, Diazinon, Chlorpyrifos, Teratogen, Embryo.*

P-62

Study the effects of tamoxifen free and tamoxifen loaded solid lipid nanoparticles (SLN) on the telomerase reverse transcriptase (TERT) and UterinOvarian-specific 44 (UO-44) genes expression in the uterus of female rats

Foroughi Sh.
Department of Clinical Biochemistry, Hamadan University of Medical Sciences, Hamedan, Iran.
Email: foroughishd@gmail.com

Introduction: An in vivo study was carried out to compare the effect of tamoxifen-loaded solid lipid nanoparticles and free drug on the Telomerase reverse transcriptase (TERT) and uterine-ovary specific gene 44 (UO-44) genes expression.

Materials and Methods: Thirty six Sprague Dawley rats aged 7 and 8 wk, weighing 200-250 gr were ovariectomised to reduce the errors due to estrogenic variation in sexual cycle. The rats were divided into six groups of six rats each. The first group comprised untreated ovariectomised rats and served as the control group 1, while the second group received 2 mg tamoxifen per kg body weight dissolved in olive oil, the third group received 2 mg/kg tamoxifen-loaded SLN, the fourth group was treated with SLN, and the fifth group received olive oil. The groups ovariectomised rats received treatment for 6 consecutive days to the animals using gastric intubations. The sixth group comprises untreated normal healthy rats and served as the control group 2. At the end of the study, the rats were scarified and examined for the genes expression.

Results: The results showed that the expression of TERT in the group treated with tamoxifen, and tamoxifen-loaded solid lipid nanoparticles, significantly decreased ($p \leq 0.001$) compared with ovariectomised control group 1. The ovariectomised control group 1 compared with the normal healthy rats showed increased significantly ($p \leq 0.001$) gene expression of TERT. While the group treated with tamoxifen showed non-significantly decreased ($p=0.058$). UO-44 gene expression, tamoxifen-loaded SLN significantly increased ($p \leq 0.001$) the gene expression compared to the ovariectomised control group 1. The results also showed that the UO-44 gene expression in group control 1 was significantly increased compared to untreated normal healthy rats and the result showed that the UO-44 gene expression in group control 1 compared with the group treated with tamoxifen were not significant different ($p=1.00$).

Conclusion: Based on the results, encapsulation of the drug in the nano-sized carriers not only did not decrease medicinal effect, but also it was increased. In the other hand the small doses of tamoxifen will show the same effects as if it encapsulated in solid lipid nanoparticles.

Key words: *Tamoxifen, SLN, TERT, UO-44, Gene expression.*

P-63

Apoptotic index (Bax/Bcl2 expression) decrease of mouse primary follicles culture on decellularized human amniotic membrane

Motamed M[1, 2], Oryan S[2], Sadr SZ[1],Moini A[3, 4], Totonchi M[5], Ebrahimi B[1], Valojerdi MR[1], Fathi R[1].

1. *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Animal Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.*
3. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Department of Gynecology and Obstetrics, Roointan Arash Women's Health Research and Educational Hospital, Tehran University of Medical Sciences, Tehran, Iran.*
5. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: rfathi79@royaninstitute.org, rfathi79@yahoo.com

Introduction: In vitro ovarian follicle culture method prepare a beneficial model in oder to study folliculogenesis and may also maintain reproduction potential of females confront premature ovarian failure causing from cancer treatments. Utilization of extra cellular matrix to support follicle morphological structure during in vitro culture is a noticeable aspect. Therefore, in this study, Decellularized Amniotic Membrane (DAM) was used because of its advantages (easy obtaining, rich ECM and growth factors) for improving in vitro follicle culture technique.

Materials and Methods: Amniotic Membrane (AM) was decellularized with trypsin EDTA. DNA quantitative and histological evaluations were performed to investigate the successful decellularization procedure. For in vitro culture, the small parts of DAM were coated on the bottom of 96-well microplates and each well was filled with 150 µl of Base Medium (BM): MEM-α +1% FSH, %1 ITS and 5% FBS. Then, isolated mouse primary follicles (90-110 µm) were individually put in each well (DAM group). Follicles which were cultured without DAM considered as control group. After nine days culture period, follicular morphology, size, viability, estradiol production and genes expression of Bax and Bcl2 were evaluated.

Results: The results revealed that the follicles cultured with DAM had improved growth and development. Viability and diameter rates in DAM group were higher than control one, also DAM group was more active in estradiol hormone production ($p<0.005$). Apoptotic index (*Bax/Bcl2* expression) in control group and

survival index (*Bcl2/Bax* expression) in DAM group had maximum expression levels respectively ($p<0.005$).
Conclusion: Higher survival index in DAM group demonstrated that DAM could improve in vitro follicular viability and high estradiol production and confirming a good functionality role. Therefore, DAM as a biological layer seems to be a suitable ECM which provide an appropriate condition as a natural system improving follicular development.
Key words: *Folliculogenesis, Extracellular matrix, Amniotic membrane, Decellularization.*

P-64
Evaluation of mitochondria distribution, ATP level and ROS of mature oocyte after vitrification of mouse ovary and three dimensional culture

Abdi Sh[1], Salehnia M[2], Hosseinkhani S.
1. *Department of Anatomical Sciences, School of Medicine, Azad University of Medical Sciences, Tehran, Iran.*
2. *Department of Anatomy, Tarbiat Modares University, Tehran, Iran.*
3. *Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: shabnam.abdi62@yahoo.com

Introduction: Invitro follicle culture techniques in combination with cryopreservation of ovarian tissue may affect mainly cytoplasmic activities such as mitochondrial function, metabolism, and intracellular signaling pathways so vitrified oocyte and follicle have been found to mature somewhat more slowly than fresh samples.
Materials and Methods: This experimental study was carried out on 7-day-old female mice (NMRI). In the first step, the ovaries were vitrified with a solution containing ethylene glycol. In the second step, the non-vitrified and vitrified ovaries were cultured in base medium α-MEM for 7 days then their morphology, hormone assay were analyzed. In third step, mechanically isolated preantral follicles were cultured in three dimensional systems in α-MEM supplemented with 5% FBS, 100mlU/ml, 1% ITS, 10 ng/ml rEGF for 12 days. At the end 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 and ultrastructure were similar to non-vitrified ovaries. The results of last 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. Most mitochondria were seen as large and homogenous aggregates in fresh oocytes compared to in vitro oocytes.
Conclusion: Our result showed that vitrification and in vitro culture of ovarian follicle could alter the distribution of mitochondria, ATP and ROS of matured oocyte in compare with in vivo group (control).
Key words: *Vitrification, In vitro maturation, Mitochondria.*

P-65
TLR3 and its signaling pathway in endometriosis

Zamani Almasi M[1, 2, 3], Moini A[2], Ramazanali F[2], Shahhoseini M[3], Hosseini E[4], Ashrafi M[2], Afsharian P[3], Aflatoonian R[2].
1. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Anatomy Department, School of Medicine ,Iran University of Medical Sciences, Tehran, Iran.*

Email: R.Aflatoonian@gmail.com; Pafshar@royaninstitute.org

Introduction: Endometriosis is a common type of chronic inflammatory disease; many studies have been shown that the expression level of inflammatory cytokines changed during endometriosis, which can affect in pathogenesis of endometriosis. TLR3 is a member of toll like receptor family which plays a critical role in innate immunity through directly recognizing exogenous and endogenous ligands and also involves in the processes of cell proliferation, survival, apoptosis, angiogenesis, tissue remodeling and repair. TLR3 after binding to its adaptor molecule, TRIF (TICAM1), leads to expression of some inflammatory genes such as IL-6 and IL-8 by NFkB pathway.
Materials and Methods: Case-control study was performed on 83 endometriosis patients whom had been confirmed by laparoscopic surgery and 93 healthy controls that had no history of inflammatory disorders or using any related drugs. All women taking part in this study along 2012-2014 were between 20-45 yr old.
Results: After DNA extraction from peripheral blood samples, Exon4 of TLR3 were amplified in two overlapped fragments by polymerase chain reaction (PCR) and products were analyzed by sequencing to determined allele and genotype frequencies. Then, RNA was extracted from tissue samples to analysis of TLR3, TICAM1, IL-6, IL-8 and NFkB mRNA expression by Quantitative Real-time PCR .In endometriosis patients higher expression of TLR3, TICAM1, IL-6, IL-8 and NFkB were observed compared to control group. These pro/anti-inflammatory factors can increase proliferation, progression, maintenance and survival of endometriotic lesions and lead more angiogenesis and neoplasia process. The two observed polymorphisms in exon 4 of TLR3 which play a critical role in its signaling pathway, rs3775291 (Missense, CTC⇒ TTC, Leu412Phe) and rs3775290 (Synonymous, TTC⇒ TTT, Phe459=), showed no significant relationship between them and occurrence of endometriosis, although the frequency of C allele (rs3775291) and T allele (rs3775290) was more in the patient group which may play a role in efficiency of TLR3.

Conclusion: Alteration in TLR3 and its signaling pathway genes may play a key role in the occurrence and maintenance of endometriosis. Although, TLR3 polymorphism in exon 4 is not effective in the pathogenesis of endometriosis, but other TLR3 mutations which affect TLR3 expression or function may involve in this disorder TLR3 is involved in the development and survival of endometriosis through NFkB pathway and it is better to investigate genetic and epigenetic changes in more samples and in the promoter region.

Key words: *Endometriosis, Toll-like receptor 3, Polymorphism, NFkB.*

P-66

A comparative analysis between day 2 and day 3 embryo transfer in IVF/ICSI: A retrospective cross-sectional study

Zamaniyan M[1, 2], Zadehmodarres Sh[2].

1. *Infertility Center, Department of Obstetrics and Gynecology, Mazandaran University of Medical Sciences, Sari, Iran.*
2. *Infertility and Reproductive Health Research Center (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: marziehzamaniyan@gmail.com

Introduction: Although the fertilization and cleavage rate of implanted embryos is about 70-90% in most patients, only a small number of embryos grown in vitro have the potential to implant. This indicates that many factors are responsible for a successful implantation, including obtaining viable embryos for transfer. This study aimed to examine the clinical results of pregnancy and implantation rates between day 2 and day 3 embryo transfer (ET) in women under the age of 40 experiencing fresh intracytoplasmic sperm injection-embryo transfer (ICSI-ET) cycles.

Materials and Methods: In a retrospective study, a total of 284 ETs were examined from March 2013 to December 2014. The transfer was done according to physician's preference, patient characteristics or number of embryos available.

Results: The data suggested that clinical (35.4% vs. 28.9%, p=0.26) or ongoing pregnancy (32.5% vs. 23.7%, p=0.11) or implantation rate (0.267±0.2 vs. 0.216, p=0.09) was slightly better and the miscarriage rate (3.1% vs. 7%, p=0.153) was slightly lower on day 3 ET vs. day 2, however, this difference was not significant. Although most of the baseline characteristics were similar between groups, the number of high-quality embryos (5.29±3.9 vs. 4.47±3.05, p=0.011) and average embryo cleavage score (2.85±0.4 vs. 2.25±0.3, p<0.001) was significantly higher in the day 3 ET in comparison to the day 2 ET.

Conclusion: A similar clinical outcome between ET performed on days 2 and 3 in women younger than 40 years undergoing fresh ICSI-ET is suggested by the results of this study.

Keywords: *Embryo transfer, Fertilization, Implantation, Pregnancy.*

P-67

Does intrauterine saline infusion by intrauterine insemination (IUI) catheter as endometrial injury during IVF cycles improve pregnancy outcomes among patients with recurrent implantation failure? An RCT

Salehpour S[1], Zamaniyan M[2, 3].

1. *Preventive Gynecology Research Center (PGRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Infertility Center, Department of Obstetrics and Gynecology, Mazandaran University of Medical Sciences, Sari, Iran.*
3. *Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran.*

Email: marziehzamaniyan@gmail.com

Introduction: Recurrent implantation failure is one of the most issues in IVF cycles. Some researchers found that beneficial effects of endometrial Scratching in women with recurrent implantation failure, while some authors demonstrated contrary results. The present study aimed to investigate the effect of intrauterine. Saline infusion as a form of endometrial injury, during fresh in vitro fertilization-embryo transfer cycle, among patients with recurrent implantation failure.

Materials and Methods: In this clinical trial study 63 women undergoing assisted reproductive technology were divided into two groups either local endometrial injury by intrauterine saline infusion during day 3-5 of the ongoing controlled ovarian stimulation cycle, or IVF protocol performed without any other intervention in Taleghani Hospital, Tehran, Iran. The main outcome measure was clinical pregnancy rates.

Results: Patients who received intra uterine saline infusion (n=20), had significantly lower clinical pregnancy numbers (1 vs. 9, p<0.05) and implantation rates (4.7% vs. 41.6%, p<0.05), compared to controls (n=39). However, there was no significant difference in miscarriage rates (9.4% vs. 8.7%, p>0.05) and multiple pregnancy numbers (1 vs. 3, p>0.05) between groups.

Conclusion: When intrauterine saline infusion as a form of endometrial injury is performed during the ongoing IVF cycles it has negative effect on reproductive outcomes among patients with recurrent implantation failure.

Key words: *Artificial insemination, Embryo implantation, Embryo transfer, Fertilization in vitro, Endometrium, Injuries, Pregnancy.*

P-68

Effects of low doses of exogenous thymoquinone on sperm motility and viability of normozoospermic men

Dashti GhR[1], Fazelian Kh[1], Golshan-Iranpour F[1], Baghazadeh Sh[2].

1. *Department of Anatomical Sciences, School of Medicine Isfahan University of Medical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Andrology Laboratory,Shahid Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: dashti@med.mui.ac.ir

Introduction: Sperm motility is a highly complex molecular process which is the result of transverse waves exist along its flagellum. Thymoquinone (TQ) is the most abundant active component isolated from black seed (Nigella Sativa). It was revealed that in-vivo administration of thymoquinone could improve spermatogenesis and increase number and motility of spermatozoa.
Materials and Methods: Twenty semen samples of normozoospermic men were washed in modified Ham's F10 medium containing albumin. 10 semen samples were used for each concentration of thymoquinone (5 and 10 µg/ml). Each sample was washed and two aliquots of it were incubated with or without considered dose of thymoquinone. Sperm motility and viability were assessed after two hours of incubation. Also, sperm motility was graded as fast- and slow-progressive, non-progressive and immotile.
Results: Both doses of thymoquinone increased the percentage of total motile and fast-progressive sperms. Administration of 10 µg/ml of thymoquinone increased the percentage of slow-progressive sperms while the dose of 5 µg/ml reduced it. The percentage of non-progressive and immotile sperms was decreased but the percentage of viable sperms was not changed after using thymoquinone.
Conclusion: Low doses of thymoquinone can increase sperm motility in culture media.
Key words: *Nigella sativa, Spermatozoa, Sperm motility, Thymoquinone*

P-69

Protective effect of melatonin on trace elements concentration and histological structure in newborn rat's kidney after exposure of cadmium chloride during pregnancy

Bahariyan J[1], Roozbehi A[1], Nikbakht J[2], Anvari M[3], Delaviz H[1].
1. *Department of Aanatomy, Yasouj University of Medical Science, Yasuj, Iran.*
2. *Department of Pharmacology, Yasouj University of Medical Science, Yasouj, Iran.*
3. *Department of Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email:jaberbaharian@yahoo.com

Introduction: One of the most risky periods of human life is the period of pregnancy. In fact Public health is depending on the health of the mother and embryo. Nowadays, with the advancement of technology and the use of more metals, heavy metal contamination is spreading. Due to this contamination, human health and especially pregnant women are threated more seriously. Cadmium is counted as a toxic metal and a known risk to the pregnancy period. Melatonin has antioxidant and protective role against oxidative stress and free radicals. This study was to investigate the effect of melatonin on trace elements concentaration and histological structure of the new born rat kidney following the toxicity caused by cadmium chloride during pregnancy.
Materials and Methods: In this study 45 Wistar rats with an average weight of 200±20 grams were used. Male rat mated with female rat with the ratio of one to three. By observation of vaginal plaque, day zero of pregnancy was recorded. Pregnant rats were allucated in 5 groups of 9 (one control, two sham and 6 expremental groups). The control group without any intervation, treatment groups were Cadmium 6 (cad6), received cadmium choloride (5 mg/kg) just on day 6 of pregnancy, Cadmium 13 (cad13), received cadmium cholorid (5 mg/kg) just on day 13 of pregnancy, melatonin 6 (me6), received melatonin (10mg/kg) on day 6 of pregnancy, melatonin 13 (me13), received melatonin (10mg/kg) on day 13 of pregnancy, cadmium-melatonin 6 (cm6) received cadmium just on day 6 and melatonin on day 6 to 20 of pregnancy, cadmium-melatonin 13 (cm13), received cadmium just on day 13 and melatonin on day 13 to 20 of pregnancy. Sham 1 group, received melatonin solvant on days 6 to 13 of pregnancy, sham 2 group, received cadmium coloride solvant on day 13 to 20. All of the injections were intraperitoenally. Cadmium accumulation and Trace elements concentration (zinc and copper) were measured in new born tars kidney by atomic absorption spectrophotometer equipment, and tissue characteristics such as number, diameters and volume of new born rats kidney glomerulus were determined.
Results: according to the results of this study, the mean number, volume and diameter of renal glomeruli in the cadmium groups (cad6 and cad13) showed a significant reduction statistically compared to the control group ($p<0.01$). Mean concentrations of mineral trace elements, zinc and copper, in the renal tissues of all newborns of cadmium groups showed a significant reduction statistically compared to the control group ($p<0.01$). Mean concentrations of mineral trace elements, zinc and copper, in the tissues of all newborns of cadmium-melatonin groups (cm6 and cm13) showed significant statistical difference compared to cadmium groups (cad6 and cad13) ($p<0.05$).
Conclusion: During pregnancy, toxic effects and oxidative stress due to cadmium, caused histological changes in the kidney tissue and it causes a deficiency of mineral trace elements, zinc and copper. This study showed that the use of melatonin play an important role in the modulation of tissue damaging effects ans trace elements reduction in the neonatal kidney which their mothers injected with cadmium choloride.
Key words: *Melatonin, Cadmium chloride, Kidney, Rats, Pregnancy, Trace elements*

P-70

Role of 3D sonography in the investigation of uterine abnormalities among infertile population: Clinical application and review of cases

Ahmadi F, Javam M.
Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: dr.ahmadi1390@gmail.com

Introduction: Accurate diagnosis of uterine abnormalities among infertile women plays an important role in infertility treatment procedures.
Materials and Methods: A review was performed within articles published at "PubMed", "Elsevier", "Google Scholar", "EBSCO", original text books and etc. to reach the aim. Lots of unique high-quality 3D sonograms were provided in this article, using the archive of infertile patients referred to imaging department of Royan institute, Tehran, Iran, between April 2012 and March 2013. Sonograms and hysteroscopy findings of patients who were booked for hysteroscopy and underwent a preoperative three dimensional transvaginal sonography at our imaging department in this period of time were gathered for each patient. Results are provided in this lecture.
Results: Lots of infertile women present at imaging centers to be evaluated for the presence of uterine cavity abnormalities. Three dimensional ultrasound scan (3DUS) is a recent non-invasive and cost-effective imaging method used to assess uterine cavity in this population. 3D sonography with the ability to generate planar reformatted sections through the uterus, allows making "coronal plane" of the uterus and its external contours. Thus, it facilitates differentiation between various types of uterine abnormalities and their effect on the fertility. Therefore, every specialist, particularly those working at the infertility treatment centers, needs to be expert in using 3D-sonography for diagnosis of uterine disorders. In this article, we discussed about the instruction of which in details and provided diagnostic criteria for each uterine lesion through unique cases of infertility.
Conclusion: As a reliable, simple, out-patient and cost-effective method, 3D sonography can reduce the indications for diagnostic hysteroscopy. Thus, it can be used as a proper helpful modality during infertility workup.
Key words: *3D-sonography, Uterus, Infertility.*

P-71
Assisted reproductive technologies and gynecological cancer: Is there any association?

Karimi-Zarchi M, Rouhi M, Abdolahi AH, Hekmatimoghaddam SH.
Shahid Sadoughi University of Medical Science, Yazd, Iran.
Email: Drkarimi2001@yahoo.com

Introduction: Infertility is as an important and common problem in couples necessitating assisted reproductive technology (ART) or drug therapy. Infertility is known as a risk factor for ovarian, breast and endometrial cancer. We aimed on evaluation of the history of primary infertility and previous ART in patients with the above-mentioned cancers.
Materials and Methods: In this retrospective study we evaluated all of the risk factors in patients with breast cancer, ovarian cancer and endometrial cancers who referred to the Gynecological Oncology Clinic in Shahid Sadoughi Hospital in Yazd, Iran from 2002 to 2012. We also investigated the history of primary infertility and ART in these patients before diagnosis of cancers.
Results: We gathered data from 92 patients with endometrial cancer, 84 patients with advanced epithelial ovarian cancer and 113 patients with breast cancer. There was history of infertility in 39.1% of patients with endometrial cancer who were obese (body mass index, BMI>29) and in 18.8% of patients with endometrial cancer and normal body mass index (BMI=25-29). ART had been performed in 7.3% of all patients with endometrial cancer. In patients with epithelial ovarian cancer, infertility was diagnosed in 28.4% and ART applied in 14.1%. Clomiphene with or without HCG and HMG was the most common drug used for patients with ovarian cancer. In patients with breast cancer, there was infertility in 16.5% and ART performed in 7.3%.
Conclusion: Although infertility was present as an important and fairly common risk factor in some patients with endometrial, ovarian and breast cancer, but some other factors may be more important, including age, BMI and the etiology of infertility. Finding the association between ART and gynecological cancers needs long cohort studies with follow-up of infertile women who get the ART or drug therapy for over 15-20 years. We think BMI and age (in addition to infertility and ART) are contributing factors for development of gynecological cancers.
Key words: *Assisted reproductive technologies, Infertility drugs, Body mass index, Breast cancers, Endometrial cancers, Infertility, Ovarian cancer, Age, Family history.*

P-72
Prevalence of *OCT1* polymorphism and its association with response to metformin in women with PCOs

Behjoo M[1], Kalantar SM[2], Miresmaeili SM[3], Dehghani Firouzabadi R[2], Shamsadini S[1], Nazem HA[1], Khademi Bami M[2], Akhavan Sales Zh[2, 4], Golzade M[5, 6], Sabbagh-Nejad Yazd S[6], Montazeri F[6], Kolahdooz P[6].
1. *Payame Noor Universiti, Taft, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Biology, Science And Art University, Yazd, Iran.*
4. *International Compus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5. *Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
6. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: ma.behjoo@yahoo.com

Introduction: Polycystic ovary syndrome (PCOs) is a complex endocrine disorder, affecting up to 15% of women of reproductive age. PCOs is a highly complex and heterogeneous disorder with significant contributions of both genetic and environmental factors. More than ten genes for relationships with PCOs

introduced and are being studied. One of these genes is *SLC22A1*. The human *SLC22A1* gene encoded organic cation transporter (OCT1). OCT1 is transporter of metformin in the liver. Metformin is widely for PCOs treatment. The aim of this study was evaluation of M420DEL (-/ GAT) polymorphism in patients with PCOs syndrome who are treated with metformin.

Materials and Methods: A total of 50 PCOs patients aged 16-45 yr with clinical phenotype formed the study group. They were administered by oral doses of metformin daily for three months. Before and after 45 days treatment with metformin blood level of LH was measured. Genomic DNA was extracted from peripheral blood and RFLP-PCR performed by using one pair primer for M420DEL polymorphism.

Result: Twenty six of the patients (52%) were heterozygous and twelve of them responded to metformin and decreased level of blood LH.

Conclusion: According to the recent results, assessment of the increasing or decreasing of the blood level of LH is not related to individual genotype for M420DEL polymorphism in *OCT1* gene.

Key word: *OCT1, M420DEL polymorphism, PCO syndrome, RFLP-PCR, SLC22A1.*

P-73

Evaluation of hydrocortisone (HC) effect on tight junction genes expression of the human fallopian tube

Zhaeentan Sh[1], Amjadi FS[1], Zandieh Z[1], Joghataei MT[1], Bakhtiyari M[1], Aflatoonian R[2].

1. *Department of Anatomical Science, Iran University of Medical Science, Tehran, Iran.*
2. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive, Tehran, Iran.*

Email: R.Aflatoonian@gmail.com

Introduction: Breakdown of the uterine epithelial cell junctions is a key event to implant a blastocyst. Ectopic pregnancy (EP) implantation junction breakdown occur outside of the uterine cavity with around 98% implanting in the fallopian tube. To overcome this problem, there is a need for a method that would increase the cells connection in appropriate sites especially in fallopian tube. Recently it has been shown that corticosteroids such as hydrocortisone (HC) increase the tight junction molecules genes expression in blood-nerve barrier. In this study, we investigated whether HC increase the tight junction genes expression in human fallopian tube cell line to prevent EP occur.

Materials and Methods: Human fallopian tube cell line (OE-E6/E7) was cultured with two concentration of hydrocortisone (50 nM, 100 nM) by three durations (24, 48 and 72 hr). The genes expression of tight junction molecules was investigated by QRT-PCR and compared to control. The candidate genes were *tight junction protein 1(tjp1)* and *claudin 4*.

Results: *Claudin 4* and *tjp1* genes expression were detected in all groups. The mean relative expression of *Claudin 4* and *tjp1* were increased in the concentration control, 50 nM and 100 nM respectively. The optimum HC treatment duration in both genes expression was 48 hr and there were significant differences between 48 hr and 72 hr of HC treatment.

Conclusion: Our findings indicated that HC significantly enhances the expression of tight junction molecules as compared to untreated cells. Therefore, HC may increase cells contact area in basal and basolateral aspect of cell membrane and cell integrity. Taken together, the HC seems to provide a useful tool for the prevention of EP.

Key word: *Ectopic pregnancy, Human fallopian tube, Hydrocortisone, Tight junction.*

P-74

Perception and experience of infertile women about sexual behavior: A sequential exploratory mixed study

Bokaei M[1], Simbar M[2], Yassini Ardekani SM[3], Alavi Majad H[4].

1. *Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3. *Department of Psychiatry, Research Center of Addiction and Behavioral Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Biostatistics, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: mah_bokaie@yahoo.com

Introduction: Sexual behavior is an important aspect of marital life and it means the combination of the concepts of sexual behavior, attitudes, experiences, actions, feelings and its thoughts. Infertility is one of the factors that could affect women's sexual behavior. The aim of this study is to explain and analyze the perceptions of infertile women's sexual behaviors and to design valid and reliable instrument to measure it.

Materials and Methods: This study (PhD Theses) is a sequential exploratory study which done in three sections: In the first section, content analysis approach was done to understand and dimensions of sexual infertile women behavior. The purposeful sampling was done with 15 infertile women and eight key informant working in Yazd Infertility Center. Data were collected using semi-structured in-depth interviews and analyzing them using content analysis approach was conducted. In order to verify the accuracy and strength of data were used Guba & Lincoln criteria (Credibility, Dependability, Comfirmability and Transferability). In the second section, with inductive- deductive approach, the questionnaire was designed based on the extract themes from qualitative section, expert panel and review of literature to measure the perceptions and experiences of the examiners and the clients towards infertile sexual

behavior. The validity of the inventory was assessed by using face, content validity assessment methods. The third section of this study was a cross- sectional study. The designed questionnaires filled out by 407 participant. Yazd Infertility Center was the location of study and purposive sampling was type of sampling. SPSS version 21 was used for data analysis. Spearman correlation, one way ANOVA, T Test were used for data analysis.

Results: Theme analysis of qualitative study led to 756 initial code, 114 final code, 29 sub category, 9 category and 5 themes, include, 1) factors between the couple, 2) medical interventions: opportunity or threat, and 3) the effect of environmental factors. The initial tool had designed by 113 statement and 5 structures. In other words, the effectiveness of environmental divided to two structures, common beliefs and influence of husband, relatives, community and education, and medical interventions divided to two structural.in face validity 4 phrase could not earn suitable score and removed. In CVR and CVI,23 phrase reduce and 77 item in 5 structure. This tool is measured with the 5 point Likert score is 77-385 point and measured perception, experience, dimensions and factors affecting sexual behavior of woman facing infertility. Reliability of tool with test re test was obtained 0.93. For Internal consistency using Cronbach's alpha and was obtained 0.809 for the total tool. Intra class correlation (ICC) was achieved 0.928 for tool. Results of the third part: In this cross-sectional study "Infertile Women Sexual Behavior Scale (SBWI)" was given to infertile women who referred to Yazd Infertility Center from October until December and finally 407 questionnaires were analyzed .The average age of infertile women who participating in the study was 30.2±5.23 yr, the average duration of marriage 7±4.22, and the mean duration of infertility diagnosis was 5.2±3.7 yr. One of the most influential factor on the sexual behavior of infertile women was Infertility treatment .

Conclusion: Infertile women's sexual behavior was explained the relationship between men and women, for "pleasure "and with important aim that means "childbearing" .The results of qualitative and quantitative data showed that intimacy and relations between spouses after diagnosis and during treatment of infertility in half of the cases was improved. Moreover "Infertile Women's Sexual Behavior Scale" Is a valid and reliable tool and can used in infertility centers for better understanding of sexual behavior of infertile women .With setting up sexual counseling classes in infertility centers, we can be expected improve better sexual life, in most infertile couple's.

Key words: *Sexual behavior, Sexual function, Infertility, Qualitative study.*

P-75
Clinical efficacy of uterine artery embolization in infertile women with uterine fibroids

Jalilian N, Hematti M.
Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: njalilian@yahoo.com

Introduction: Uterine leiomyomas (fibroids or myomas) are the most prevalent benign tumors in pelvic region in females, which are usually reported in pre-menopause women. They originate from the smooth muscle cells of the myometrium and result in symptoms such as abnormal uterine bleeding and pelvic pain or pressure. We decided to evaluate the efficacy of uterine artery embolization (UAE) in infertile women with symptomatic uterine fibroids.

Materials and Methods: In this descriptive study, 65 premenopausal patients, without considering the fibroids size and its location, were treated by bilateral UAE. At baseline and after 3, 6, and 12 months MRI was obtained to determine the uterine length and fibroid diameter. In addition, symptoms of the patients were documented at these follow-up schedules. SPSS version 20 was used for statistical analysis.

Results: UAE was successful in 62 (95.4%) cases. Complete infarction rate of the fibroid was 83.1%. After 12 months, the uterine length showed a decrease of 55.7% (mean of 9.4 cm) and the diameter of the dominant fibroid revealed a decrease of 52.1% (mean of 3.4 cm). Menorrhagia improved in 45 cases (91.8%), abdominal mass in 24 cases (82.28%), urinary symptoms in 17 cases (85%), pelvic pain in 21 cases (84%), and dysmenorrhea in 25 cases (80.6%). Final follow-up was performed after one year and complete infarction of the fibroma was demonstrated in 49 patients (83.1%). Two cases achieved successful pregnancy in the one year follow-up period. Five patients developed post-embolization syndrome which necessitated admission to the hospital. Twenty-two patients presented and complained of pain for which outpatient pain management was performed.

Conclusion: UAE was a successful treatment for uterine fibroids that preserved the uterus and required short hospitalization and recovery.

Key words: *Uterine artery embolization, Uterine fibroma, Complication.*

P-76
Intravenous immunoglobulin (IVIG) modulates regulatory T cells and improves pregnancy outcome in patients with repeated implantation failure (RIF)

Abdolmohammadi-Vahid S[1, 2], Ghaebi M[1, 2], Ahmadi M[1, 2], Abdollahifard S[3], Danaii Sh[4], Mousapour P[4], Heidari L[4], Hazrati F[4], Seyyed Hosseini R[4], Nouri M[5], Yousefi M[1, 2].

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Department of Transplantation, Tabriz University of Medical Sciences, Tabriz, Iran.*
4. *Gynecology Department, Eastern Azerbaijan ACECR ART center, Eastern Azerbaijan branch of ACECR, Tabriz, Iran.*
5. *Reproductive Biology Department, Tabriz University of Medical Sciences, Tabriz, Iran.*

Email: Svahid1371@ymail.com

Introduction: Repeated implantation failure (RIF) is defined when embryos of good quality fail to implant following several in vitro fertilization (IVF) cycles. Regulation of maternal immune system in favor of regulatory T cells (Tregs) is essential to prevent the embryo rejection. Any abnormalities in the frequency or function of Tregs resulted in pregnancy loss. Immunotherapy with IVIG has been introduced over the past decade to improve IVF success rate and it was recommended for treatment of recurrent miscarriages. However, the molecular and cellular mechanisms underlying IVIG effects are not completely understood.
Materials and Methods: A group of 44 RIF patients with cellular immune abnormalities were included in this study. 24 out of 44 women received IVIG and 20 out of 44 did not receive IVIG as control group. A dose of 400mg/kg IVIG was administered 1-2 days before embryo transfer by intra- venous infusion with blood samples drawn before each infusion. Additional blood sample were taken at day 15. Frequency of Treg and TGF-β, IL-10 and Foxp3 proteins and mRNA levels were determined using flowcytometry, ELISA and Real time PCR respectively. Pregnancy rate was checked by the serum β-hCG level, 16 days after embryo transfer.
Results: Our results showed significantly high number of Tregs in RIF patients following treatment of IVIG. Moreover TGF-β, IL-10 and Foxp3 proteins and mRNA levels were increased using IVIG therapy. Pregnancy rate in IVIG treated subjects (52%) was significantly higher than untreated group (27%).
Conclusion: Our findings suggest that the rate of successful pregnancy increased after IVIG administration particularly in those with cellular immune abnormalities through T reg-type responses.
Key words: *Intravenous immunoglobulin, Repeated implantation failure, T reg, Treatment.*

P-77

Effect of intravenous immunoglobulin on Th1 and Th2 lymphocytes and improvement of pregnancy outcome in recurrent pregnancy loss (RPL)

Abbaspour Aghdam S, Ahmadi M, Yousefi M.
Stem Cell and Regenerative Medicine Institute, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: abaspour.sanaz@gmail.com

Introduction: Recurrent pregnancy loss (RPL), as a global issue, is increasing all over the world and is classically determined as 3 or more pregnancy losses before the 20th wk of gestation and it is associated with genetic, anatomic, hormonal, infectious and immunological abnormalities while almost 50% of cases are idiopathic. RPL Women with elevated NK cells frequency and function through pregnancy, suffer from RPL. Treatment with IVIG in RPL patients especially with elevated rate of NK cells and Th1 cells is effective and improves the pregnancy outcome.
Materials and Methods: Totally, 44 women, 32 with a history of RPL and 12 as a control group enrolled in the study. The frequency of Th1 and Th2 lymphocytes, transcription factors and serum level of related cytokines were assessed by flowcytometry, real-time PCR and ELISA, respectively pre and post-treatment with IVIG.
Results: A significant reduction in Th1 lymphocytes frequency, transcription factor expression and cytokine levels were observed in IVIG treated group while all the above parameters increased about Th2 lymphocytes. Th1/Th2 ratio decreased significantly ($p \leq 0.0001$) at the end of treatment and 28 out of 32 (87.5%) women in IVIG group had live birth in comparison with 5 out of 12 (41.6%) in untreated group.
Conclusion: IVIG as a good therapeutic option, is able to enhance the success rate of pregnancy through a shift in Th2 responses and it is effective in treatment of reproduction failures especially in subject with immune cell abnormalities and increased NK cell level and function.
Key words: *Recurrent pregnancy loss, Intravenous immunoglobulin G, Treatment, Th1/Th2 ratio.*

P-78

Variations in T-helper 17 and regulatory T cells during the menstrual cycle in peripheral blood of women with recurrent spontaneous abortion

Sereshki N[1], Gharagozloo M[1], Ostadi V[1], Ghahiri A[2], Roghaei MA[2], Mehrabian F[2], Andalib AR[1], Hassanzadeh A[3], Hosseini H[4], Rezaei A[1].
1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Department of Obstetrics and Gynecology, Al-Zahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
3. *School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran.*
4. *Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: rezaei@mui.ac.ir

Introduction: Disorders in immune system regulation may result in pregnancy abnormalities such as recurrent spontaneous abortion (RSA).
Materials and Methods: In this case control study, 25 women with RSA and 35 healthy, non-pregnant women were enrolled. The percentage of Th17 and Treg cells in participants peripheral blood were determined by flow cytometry.
Results: The percentage of Th17 cells and their related cytokines in serum (IL-17A) were higher in the proliferative and secretory phases of the menstrual cycles of RSA women compared to the control women. However, a lower percentage of Treg cells and their related cytokines in serum, transforming growth factor (TGF) β1 and interleukin (IL)-10 were detected in the proliferative but not the secretory phase of the URSA group. The ratio of Th17/ CD4+ Treg was higher in the RSA group than the control group. We observed an increased ratio of Th17/ CD4+ Treg during the proliferative and secretory phases in RSA women.
Conclusion: The imbalance between Th17 and Treg cells during the proliferative phase of menstrual cycles

in the RSA group may be considered a cause for spontaneous abortion.

Key words: *Regulatory T cells, T helper 17, Menstrual cycle, Pregnancy.*

P-79

Change of T helper (Th) 17 cells in patients with unexplained recurrent miscarriage after lymphocytes immunization therapy (LIT).

Rafiee M[1], Ghahiri A[2], Maracy MR[3], Kouhpayeh Sh[1], Rezaei A[1].

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Department of Gynecology and Obstetrics, Al-Zahra University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
3. *Department of Community Medicine, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: mitra_rafiee@yahoo.com

Introduction: Two to five percent of women, experience Recurrent Miscarriage (RM) during the first trimester of pregnancy. The etiology of recurrent miscarriage remains unknown in the majority. It has been postulated that immunologic disturbance may be responsible for these cases of RM. Lymphocytes Immunization Therapy (LIT) is an effective treatment for RM women. However the exact mechanisms of immunotherapy with paternal lymphocytes have yet to be elucidated. One of the beneficial effects of Lymphocytes Immunization Therapy include specific and non-specific T cell suppression. On the other hand, an increase in Th17 cells, IL-17 and IL-23 levels has been found in the peripheral blood and decidua of women with RM in comparison to normal pregnant women.

Materials and Methods: The expression levels of CD4 and IL-17 in Th17 cells, were evaluated with flow cytometry pre- and 3 months post-immunotherapy in RM patients treated with paternal lymphocytes.

Results: LIT decreased the frequency of Th17 cells in peripheral blood of RM patients after treatment ($p \leq 0.05$).

Conclusion: Considering RM patients have a higher Th17 cells in peripheral blood, LIT may be considered as an effective therapeutic approach to treat RM patients with decrease the frequency of Th17 cells in peripheral blood of these women.

Key words: *Recurrent miscarriage, T helper 17, Lymphocytes Immunization Therapy.*

P-80

The efficacy of intravenous immunoglobulin (IVIG) in pregnancy success and modulation of Th17 responses in women with recurrent implantation failure (RIF)

Ghaebi M[1], Abdolmohammadi-Vahid S[1], Ahmadi M[1], Nikmaram A[1], Farzadi L[2], Ghasemzadeh A[2], Hamdi K[2], Nouri M[3], Yousefi M[1].

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Gynecology, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Department of Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran.*

Email: mahnaz.ghaebi@yahoo.com

Introduction: Recurrent implantation failure (RIF) is defined as failure to achieve pregnancy following at least three cycles of in vitro fertilization (IVF). There is growing evidence that immunologic factors may account for most of the cases of implantation failure. Th17 has emerged as a risk factor threatening pregnancy in recurrent implantation failure. In recent years, immunotherapy has been introduced into IVF processes to achieve a better outcome. Despite the vast usage of IVIG in many clinical disorders, the underlying mechanisms are not clear understood.

Materials and Methods: 44 women with a history of RIF and abnormalities in immune cells were included in our study. 24 out of 44 women received IVIG and 20 out of 44 did not receive IVIG as control group. Blood samples were collected to measure frequency of Th17 cells, IL-17 and IL-23 mRNA expression and cytokine secretion. A dose of 400mg/kg IVIG was administered 1-2 days before embryo transfer by intravenous infusion with blood samples drawn before each infusion. Additional blood sample were taken at day 15. Pregnancy rate was checked by the serum β-hCG level, 16 days after embryo transfer.

Results: No statistically significant decrease was observed in Th17 frequency and cytokines secreted by Th17 including IL-17 and IL-23 following treatment with IVIG. However pregnancy rate in IVIG treated subjects (52%) was significantly higher than untreated group (27%).

Conclusion: These data suggest that pregnancy outcome might be improved by IVIG through effects on the other immune cell populations including NK cells and Tregs.

Key words: *Recurrent implantation failure, IVIG, Pregnancy, Th17.*

P-81

Cost benefit analysis of infertility treatment by IVF based on WTP approach in Iran

Darvishi A[1], Goudarzi R[2], Habibzadeh V[3], Barouni M[4].

1. *Health Services Management Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran.*
2. *Modeling in Health Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran.*
3. *Afzalipour Clinical Center for Infertility, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran.*
4. *Social Determinants of Health Research Center, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran.*

Email: alidarvishieco@gmail.com

Introduction: Infertility of couples has become an important issue in the world today. In vitro fertilization (IVF) is an assisted reproductive technology that is usually very costly. Due to the high prevalence of infertility in Iran and the high costs of infertility treatments, analysis the economic issues of this scope seems important.
Materials and Methods: This study is a cross-sectional descriptive analytical study and a kind of full economic evaluation studies in health care system. The costs per capita of one cycle of IVF were calculated according to the treatment protocols, tariffs of 2016, and medical information record of patients. Willingness to pay (WTP) of people was measured by Contingent valuation method (CVM) using a researcher-made questionnaire from March to June 2016 in Kerman.
Results: The costs per cycle of IVF treatment from the first visit to the last stage was estimated 60,897,610 IRR. The amount of WTP for this treatment method was estimated at 29,535,410 IRR for each treatment cycle. Regarding the costs and outcomes of this intervention, it was found that IVF treatment was not cost-benefit and had a negative net benefit.
Conclusion: The attitude and viewpoint in policy-making for the treatment of infertility is of great importance. If we consider the absolute and short-term economic vision of funding issue for the treatment of infertility, as the results of this study showed, investment on IVF method has no positive net benefit. But if the long-term economic vision is considered, the child born to this method of treatment is enable to become a productive economy-maker in the future. So it is required that policy-makers pay more attention to this issue in the current period that we require population growth in Iran.
Key words: *Cost benefit analysis, In vitro fertilization, Willingness to pay, ART.*

P-82
Intravenous immunoglobulin (IVIG) modulates Th17 cells and improves live birth rate in patients with recurrent spontaneous abortion

Eghbal-Fard S[1], Ahmadi M[1], Dolati S[1], Nouri M[2], Babaloo Z[1], Farzadi L[3], Jadidi Niaragh F[1], Yousefi M[1].
1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Department of Gynecology, Tabriz University of Medical Sciences, Tabriz, Iran.*

Email: mehdi_yusefi@yahoo.com

Introduction: Recurrent spontaneous abortion (RSA) is a growing problem in the Iran, particularly among women over 30 years of age. In these women, RSA occurs with both natural and artificial fertilization techniques. There is increasing evidence that immunologic factors play an important role in RSA. Th17 cells are recently proposed as new risk factors in RSA. Higher level of IL-17 has been reported in women with unexplained RSA compared with women with normal pregnancies Intravenous immunoglobulin G (IVIG) was shown to cause immunomodulation in RSA patients.
Materials and Methods: 38 RSA subjects with cellular immune abnormalities were included and peripheral bloods was drawn upon positive pregnancy test. On the same date, IVIG, 400 mg/kg, was administrated intravenously and continue every 4 wk through 28-30 wk of gestation. 12 RSA patients with abnormal cellular immune profile were included as IVIG untreated group. We investigated IVIG effect on Th17 cells frequencies and cytokine secretions and pregnancy outcome in RSA patients before and after treatment.
Results: IVIG treatment significantly reduced the frequency of Th17 cells from 3.94±1.12% to 1.83±0.56%. Moreover, significant reduction in RORγt and IL-17 mRNAs and cytokine secretions (IL-17 and IL-23) were observed in IVIG treated patients. Pregnancy outcome in IVIG treated subjects (87.5%) was significantly higher than untreated group (41.6%).
Conclusion: Our findings shed more light on the mechanisms of IVIG immunomdulatory effects and introduced IVIG as a promising therapeutic approach in RSA patients with cellular immune system abnormalities.
Key words: *Recurrent spontaneous abortion, Intravenous immunoglobulin G, Treatment, Th17.*

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Is estradiol level predictive for egg donation outcome?

Taheri F, Borzouie Z, Eftekhar M, Mangoli E.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: taherifatemeh86@yahoo.com

Introduction: Gonadotropin therapy plays an important role in ovarian stimulation for infertility treatments. As a result, an amplified elevation of estradiol (E_2) in serum levels occur. Although estradiol levels remain an important part of monitoring in most IVF programmes, the effect of E_2 level on egg donation cycles outcome has not been adequately assessed.
Materials and Methods: A retrospective analysis of 134 consecutive fresh oocyte donation cycles was performed in an assisted reproductive technique (ART). A serum E2 level (peak E2) was obtained from all oocyte donors on the morning of HCG administration. Patients were designated into three groups based on peak E_2 (Group I, <1500 >3000 pg/ml). A comparison among groups was made regarding, fertilization and chemical pregnancy rates.
Results: The result of this study shows the higher outcome in Group II of patients in comparison of other groups. However the data was not significant ($p \leq 0.05$). Fertilization rate were (63±21, 75±17, 58±12%) and chemical pregnancy rates were 22.9, 33.3 and 26.7% respectively.

Conclusion: Serum E_2 level may be a predictor of ART outcome for egg donation cycles.

Key words: *ART, Egg donation, Estradiol, Chemical pregnancy.*

P-84

Effect of therapeutic yoga in reducing anxiety and depressive symptoms and improving quality of life in infertile women waiting for IVF

Mousavi Nik M[1], Birashk B[2], Assari A[2].

1. *Department of Art and Architectur, Faculty of Clinical Psychology, Tehran, Iran.*
2. *Mental Health Research Center, Tehran Institute of Psychiatry, School of Behavioral Sciences and Mental Health, Iran University of Medical Sciences, Tehran, Iran.*

Email: maryammousavinik@gmail.com

Introduction: Infertility and its treatment is a complex and prolonged crisis and it is a stressful condition that creates a heavy psychological and an emotional trauma for the couples. Depression and anxiety are considered as one of the main psychological disorders associated with infertility and it may significantly affect the life of infertile individuals, their infertility treatment, and follow-up. Yoga has a method of stress management tool that can assist in lessening depression and anxiety disorders. Yoga is a method of mind-body fitness that includes a combination of muscular activity and mindful focus on awareness of the self, the breath and energy. Therapeutic yoga is the application of yoga positions and practice to the treatment of health and well being conditions and involves instruction in yogic practices and teachings to prevent reduce or improve structural, physiological, psychological and emotional.

Materials and Methods: This research was a semi experimental study using control group also pre-test, post- test and follow-up. To assess the level of anxiety, depression and Quality of Life, thirty infertile women waiting for IVF were selected randomly and administered Cattle questionnaires for surveying anxiety, Beck Depression Inventory-II (BDI-II) for measuring depression level and fertility-related quality of life (Ferti-QoL) for evaluating the Quality of Life. All subjects were randomly assigned to experimental group (n=15) and control group (n=15). Subjects in experimental group were offered participation in 3-months yoga programs. Pre-assessment was carried for both the group at the beginning of the intervention. Post assessment was done for both the groups after completing Therapeutic yoga intervention. Third assessment was following up assessment, which was done, carried at 5th wk after post-assessment .

Results: Results indicates that therapeutic yoga was effective in reducing anxiety and depressive symptoms. Furthermore therapeutic yoga improving quality of life in experimental group from pre to post assessment and follow up.

Conclusion: Findings indicate that therapeutic yoga is a useful technique to alleviate of anxiety and depressive symptoms and improving quality of life among infertile women.

Key words: *Therapeutic yoga, Anxiety, Depressive symptoms, Quality of life, Infertile women, IVF.*

P-85

Effect of 4977 bp mitochondrial genome deletion on implantation stage in women undergoing IVF

Ghanami Gashti N[1], Salehi Z[2], Mashayekhi F[2], Karimi Karimlo F[2], Zahiri Sorouri Z[3], Bahadori MH[4].

1. *Department of Anatomical Sciences, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
2. *Department of Biology, Faculty of Sciences, Guilan University, Rasht, Iran.*
3. *Reproductive Health Research Center, Obstetrics and Gynecology Department, Alzahra Hospital, Guilan University of Medical Sciences, Rasht, Iran.*
4. *Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.*

Email: nasringhanami@gmail.com

Introduction: Oxidative stress is involved in the physiological acting of the female reproductive organs and it may influence the outcome of assisted reproductive technology (ART). It affects both implantation and early embryo development which determines a successful pregnancy. Oxidative stress can damage mitochondrial DNA (mtDNA). One of these damages is 4977 bp deletion in mtDNA or common deletion that embraces some genes of mitochondrial complex I, IV and V and six tRNA genes. Defective respiratory chain enzymes encoded by deleted mtDNA may further enhance free radical production and cause more oxidative damage. Deletion in mtDNA of leucocytes may increase systematic oxidative stress.

Materials and Methods: In order to detect this deletion, blood samples of 171 women undergoing IVF and 90 controls from Northern Iran were prepared. After total DNA extraction, Gap polymerase chain reaction (Gap PCR) was performed.

Results: We observed 4977 bp deletion in 59.65% of women undergoing IVF, while 6.66% of controls had this deletion (OR= 20.69, 95% CI= 8.56-50.03, $p<0.0001$). Moreover, 57.14% of IVF negative cases had 4977 bp deletion while 63.64% of IVF positive cases had this deletion (OR= 0.76, 95% CI= 0.40-1.43, p= 0.39). We observed high frequency of mtDNA 4977 bp deletion in women undergoing IVF compared to control cases. Although, there was no significant difference in deletion frequency between IVF negative and positive cases.

Conclusion: In the present study, it has been demonstrated that infertile women undergoing IVF had high frequency of common deletion in leucocyte mtDNA compared to control cases. This deletion in leucocyte mtDNA may increase ROS production in these cells and cause systematic oxidative stress. But in

our study, there was no significant difference in distribution of 4977 bp deletion between IVF negative and IVF positive cases.

Key words: *IVF, Implantation, Oxidative stress, mtDNA deletion, ART.*

P-86

Evaluation of Pro12Ala polymorphism in the peroxisome proliferator activated receptor gamma (*PPAR-γ*) gene and metformin metabolism in patients with polycystic ovary syndrome (PCOS)

Jafari F[1], Miresmaeili SM[1], Kalantar SM[2].

1. *Department of Biology, Science and Art University, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductivev Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fatemehjafari175@gmail.com

Introduction: Polycystic ovary syndrome (PCOS) is a common hormonal disorder among girls and women due to endocrine and ovulation disorder during their reproductive years. There are several genes linked to PCOS; the *PPAR-γ* gene is expressed in the fat and involved in glucose homeostasis, adipocyte differentiation, PCOS pathogenesis and enhancing the action of insulin in insulin-sensitive tissue by increasing glucose uptake adipose tissue. PPAR-γ is a transcription factor that Pro12Ala polymorphism reduces its transcriptional activity. Metformin decrease insulin in PCOS patients and improve ovulation.

Materials and Methods: A total of 100 reproductive-aged women were included in this case-control study were diagnosed as a PCOS based on Rotterdam criteria and 100 healthy women with no evidence of PCOS were recruited as controls. the plasma levels of follicle-stimulating hormone (FSH), and luteinizing hormone (LH) was evaluated before and 45 days after metformin consumption in patients. The case and control group genotyped using the technique PCR-RFLP for Pro12Ala polymorphism.

Results: After genotyping, there was not any significant difference in patient and control groups. In patient LH, FSH, and testosterone levels was significance difference pre and post treatment with metformin, but there is no correlation between genotype and response to metformin (p=0.59).

Conclusion: The ovulatory response to treatment with metformin and its interaction with changes in genetic or modifiable factors requires further studies.

Key words: *Denotyping, LH, FSH, Metformin, PCR-RFLP.*

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The effects of Val34Leu polymorphisms in coagulation factor XIII gene on recurrent pregnancy loss in Iranian women

Isazadeh A[1], Hajazimian S[1], Mohammadoo-Khorasani M[2].

1. *Department of Genetic, Tabriz Branch, Islamic Azad University, Tabriz, Iran.*
2. *Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: Isazadeh.Alireza@yahoo.com

Introduction: Recurrent pregnancy loss (RPL) is a heterogeneous condition consisting of three or more consecutive abortions occurring before 20 weeks of gestation. One of the clotting factor genes encodes factor XIII (FXIII), which is involved in fibrin formation. The most common polymorphism in the FXIII genes is the conversion of G to T in exon 2 (val34leu) of the FXIIIA gene, which leads to the substitution of valine with leucine. The objective of this study was to investigate the association between RPL and FXIII val34leu polymorphisms in a sample population of Iranian Azeri women.

Materials and Methods: A prospective case-control study was performed on a cohort of 310 RPL patients and 290 healthy controls. DNA was extracted from the whole blood and fragments of the Val34Leu polymorphism were amplified by polymerase chain reaction (PCR), followed by DNA sequencing. Genotyping was performed using the Sequenom MassArray system.

Results: The genotype frequencies of FXIII in the case group were 60.64% GG, 34.83% GT, and 4.41% TT, whereas the frequencies in the control group were 58.96% GG, 36.5% GT, and 4.48% TT. T allele frequencies in the case and control groups were 78.06% and 21.93%, respectively, and G allele frequencies were 77.24% and 22.75%, respectively.

Conclusion: No significant association was observed between the Val34Leu polymorphism and RPL among Iranian Azeri women.

Key words: *Val34Leu, FXIII, Polymorphism, Recurrent pregnancy loss.*

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Effects of oocyte vitrification on expression of HSP70 gene in ovine embryos and oocytes

Farmani N[1], Barati F[1, 2], Kadivar A[1, 2], Ahmadi E[2].

1. *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*
2. *Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran.*

Email: naghmeh.farmani@yahoo.com

Introduction: The Gamete and embryo cryopreservation is the most important in order to increase the economical and genetical valuation and breeding program progression. The ovum freezing and banking crucial in preservation of the endangered species. Freezing is performed to the three slow (Slow) and ultra-fast (Rapid) and (vitrification) ways. Vitrification removed many of problems related to cryopreservation of oocytes and embryos. On the hand, some problems related to in vitro produced embryos may indicate any alteration in embryonic genome transcription. The aim of the present study was to investigate whether oocyte vitrification may alter

expression of a gene that can change following environmental stress, i. e., HSP70 or not.

Materials and Methods: A total of 120 immature germinal vesicle stage ovine cumulus oocyte complexes (COC) were retrieved from abattoir collected ovine ovaries. The COCs were subjected to vitrification in HTCM based (with 20% FBS) media as V1: DMSO (10%), ethylene glycol (10%) for 30 min, V2: DMSO (10 %), ethylene glycol (10%) and 0.5 M sucrose immediately left on the cryotop device and immersed within liquid nitrogen. At least after 48 hrs of vitrification, the oocytes were warmed in warming solutions as W1: basic medium with 1 M sucrose, W2: basic medium and 0.5 M sucrose and W3 basic medium with 0.25 M sucrose, each of them for 5 min. The vitrified-warmed COCs (n=60) and fresh COCs (n=60) were subjected to routine IVM, IVF and IVC procedures of the laboratory with SOF based medias. The developmental stages of oocytes were compared and the expression rate of HSP70 to average of β-actin and B2m genes expressions were compared between blastocysts and oocytes before and after vitrification.

Results: The results of the study showed the impact of vitrification of germinal vesicle stage oocytes on the next developmental competence of the respected embryos in all stages ($p<0.05$). The expression of HSP70 was significantly different between oocytes and blastocysts; however the vitrification of immature ovine oocytes did not affect the expression rate of HSP70 in oocytes the respective blastocysts.

Conclusion: In conclusion, vitrification has no effect on the vitrified ovine immature oocytes and the next developed blastocysts.

Key words: *Ovine COCs, Vitrification, HSP70, Blastocysts, Oocyte.*

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Association of G4845T polymorphism in *IL-1A* gene with azoospermia

Zamani-Badi T, Karimian M, Nikzad H.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: t.zamani@yahoo.com

Introduction: Spermatogenesis is a dynamic process that finally leads to the production of mature sperm. A balance between proliferation and apoptosis of cells is necessary for successful spermatogenesis and cytokines are involved in this process. Interleukin-1 gene family promotes the expression of genes contributed in cell survivaland proliferation. IL-1a is a member of this family that is considered as a potential growth factor for spermatogonia. Any changes such as genetic polymorphisms in the sequence of these genes may be related to male infertility.

Materials and Methods: In a case-control study, 50 fertile and 51 infertile men were examined. All of infertile men were classified as azoospermia. 2 ml blood was collected from each fertile and infertile men. After DNA extraction, genotypes of samples at G4845T location were determined by PCR-RFLP method. To confirm the PCR-RFLP procedure, some samples were sequenced, randomly. Finally, statistical analyses performed by SPSS software.

Results: The data showed a significant association of homozygous TT (OR=5.63, 95% CI=1.56-20.31, p=0.0084) with azoospermia. Carriers of T (GT+TT) were at a high risk for azoospermia (OR=3.19, 95% CI=1.40-7.24, p=0.0056). Allelic analysis showed that the T allele was associated with azoospermia (OR=1.97, 95% CI=1.15-3.37, p=0.0138).

Conclusion: This study showed that G4845T polymorphism is a riskfactor for male infertility in our study population. This association shows that the *IL-1A* G4845T transversion may be a potential biomarker for male infertility, and it needs to complementary investigations in future studies.

Key words: *Male infertility, Azoospermia, IL-1A gene, G4845T polymorphism.*

P-90

Association of polymorphisms in *PRM1* and *PRM2* genes in Iranian infertile men with asthenozoospermia

Nabi A, Khalili MA, Moshrefi M, Sheikhha MH, Zare E.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Science, Yazd, Iran.
Email: khalili59@hotmail.com

Introduction: Numerous genetic factors are involved in human male infertility, but the main gene, related to idiopathic infertility is unknown. Protamines are DNA packaging protein in sperm. Any abnormality in protamines gene and their expression may result to male subfertility and infertility. This study aim to evaluated the polymorphisms of protamine-1 and 2 (*PRM1* and *PRM2*), as the important infertility candidates, in a group of Iranian asthenozoospermia men.

Materials and Methods: In this case-control study, test samples were corresponding to 50 asthenozoospermia infertile men who referred to Research and Clinical Center for Infertility, Yazd, Iran. The control group was compromised from 50 men who had referred for infertility treatment with female related infertility problems. Semen samples were analyzed under the standard of World Health Organization (WHO 2010). The patients' venous blood was collected, by using phlebotomy technique. DNA samples were extracted from total leukocyte blood. DNA sequence amplification was performed using four *PRM1* and *PRM2* primers, designed from 5' to 3' flank regions. Then *PRM1* and *PRM2* gene sequences was screened in search of potential mutations. The data was evaluated with SPSS.

Results: Totally nine SNPs were detected in our study. Three of them were corresponded to *PRM1* and six to *PRM2*. The defined SNPs in *PRM1* were 102G>T, 49C>T which were not reported previously and

139C>A (rs737008, Amino acid:47). In *PRM2*, 248 C>T genotype was new and five others SNPs were reported previously including rs545828790, rs115686767, rs201933708, rs2070923 and rs1646022. The allele frequency of all upper mentioned SNPs in asthenozoospermia men was higher than control group but this difference was not significant. Some of them were previously reported in protamines of infertile or azoospermiamen but not asthenozoospermia patients, and three of them were reported for the first time in this study.

Conclusion: In our selected Iranian population, these nine SNPs were present, but their association was not significant in asthenozoospermia patients compared to healthy ones, that may be due to limited tested population. So, we proposed more extensive study, to associate these SNPs, as molecular markers for detecting asthenozoospermia infertile men.

Key words: *Protamine, Asthenozoospermia, Polymorphisms, SNP.*

P-91
Endometriosis influence on fertility

Denys T, Gayvoronskaya S.
Department of Obstetrics and Gynecology No. 2, Kharkiv National Medical University, Kharkiv, Ukraine.
Email: collapse17@mail.ru

Introduction: Endometriosis is the female's disease of reproductive age in the context of excrescence of the mucosa covering the uterus (endometrium). Within this disease endometrium goes beyond the uterus and locates on the pelvic organs: ovaries, fallopian tubes, cervix, vaginal walls and sometimes rectum or urinal bladder. In the clinic of endometriosis main symptoms are pain and infertility. The prevalence of endometriosis among females worldwide amounts to 7-10% and among infertile females the incidence of endometriosis amounts to 20% up to 50 % that proves the research relevance. to examine the results of the survey for antibodies to Thomsen-Friedenreich antigen, hormonal analysis, instrumental methods of diagnosing women with endometriosis and to determine the influence of this data on fertility.

Materials and Methods: The experimental group consists of 30 women with endometriosis, suffering infertility. Control group consists of 10 somatically healthy women. All the women were of the same age (25-40 years). The life anamnesis, somatic, obstetric and gynecological histories analysis were made. Endometriosis was diagnosed according to clinical symptoms (pelvic pain syndrome, abnormal uterine bleeding), data of uterovaginal, ultrasound examination and histologically confirmed. The hormonal state of all patients was examined; follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol on 2-7 days of menstrual cycle. Patients were examined for antibodies to the Thomsen-Friedenreich antigen (T-antigen). In addition, all the experimental group women underwent hysteroscopy, being visually suspected for having adenomiosis followed by biopsy. 15 patients (50%) underwent diagnostic laparoscopy with chromosalpingography and following with endometriotic heterotopy biopsy.

Results: During the hormonal examination 18 women (60%) had an increase of the estradiol level (191.2±12.8 pg/ml at the rate 12.5-166.0 pg/ml, $p<0.05$). 7 (23.3%) women had an increase of the FSH level (14.2±0.4 mIU/ml at the rate 1.3-9.9 mIU/ml, $p<0.05$) and decrease of the LH level (0.95±0.2 mIU/ml at the rate 1.67-15.0 mIU/ml, $p<0.05$). 9 women (30%) had an occlusion of the fallopian tubes, as a result of heterotopias' invasion during the laparoscopy with chromosalpingography, 6 women (20%) had an obliteration of the fallopian tubes because of peritubal adhesions. All patients' diagnosis of endometriosis were confirmed during histological examination. 25 women (84%) of experimental group had antibodies to T-antigen. There were no abnormalities detected during such hormonal and instrumental examinations in the control group. Also, they had no antibodies to the T-antigen.

Conclusion: Based on the received data, we can consider that occurrence of infertility during the endometriosis depends both on the changes of hormones and on the fallopian tubes patency. The detection of antibodies to the Thomsen-Friedenreich antigen within infertile patients demonstrates high specificity of endometriosis diagnosing.

Key words: *Endometriosis, Infertility, Thomsen-Friedenreich antigen.*

P-92
Investigating the association of promotor polymorphisms of *Fas* and *FasL* genes with endometriosis

Akhavan Sales Zh[1], Sheikhha MH[2], Seifati SM[3], Tabibnejad N[3], Shamsi F[4], Tahoori MT[1].
1. *Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
4. *Department of Biostatistics and Epidemiology, Health College, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: dr.zhima@live.com

Introduction: Endometriosis is a gynecological problem described by the existence of endometrial tissue outside the uterine cavity affecting 6-10% of women in the age of reproduction. Regarding the effect of genetic and apoptosis in pathogenesis of endometrium, in this study, we evaluate the effect of *Fas* and *FasL* polymorphisms on the risk of endometriosis in a sample of Iranian people.

Materials and Methods: A total of 112 women with proven endometriosis and 110 women who had no

evidence of endometriosis were selected as case and control group respectively. *Fas* -670 A/G and *Fas*L -844 C/T polymorphisms were assessed in extracted DNA samples using polymerase chain reaction-restricting fragmentation length polymorphism. The effect of gene variation on endometriosis was evaluated.
Results: Regarding *Fas* -670 the frequency of AA genotype was 39.3% and 40% in endometriosis and control group, respectively. We found that the increased risk of endometriosis is associated with *Fas* -670 AG and *Fas* -670 GG genotype when compared to *Fas* -670 AA (OR=1.06 and 1.07 respectively) however the difference was not statistically significant (p>0.05). On the subject of *FasL* -844, the CC distribution was 61.6% in case group versus 68.2% in controls. *FasL* -844 CT and *FasL* -844 TT were also correlated with higher endometriosis occurrence with no significant differences (OR=1.13 and 1.51 respectively) (p>0.05).
Conclusion: We found no significant evidence for the association between *Fas* and *FasL* gene polymorphisms and endometriosis. Therefore, it seems that the evaluated gene variants are not involved in the pathogenesis of endometriosis in Iranian population.
Key words: *Endometriosis, Fas, FasL, Polymorphism.*

P-93
No association of promotor polymorphisms of *Fas* and *FasL* genes with different endometriosis stages

Akhavan Sales Zh[1], Sheikhha MH[2], Seifati SM[3], Tabibnejad N[3], Tahoori MT[1].
1. *Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*

Email: dr.zhima@live.com

Introduction: Endometriosis can be classified into four stages (I-minimal, II-mild, III-moderate, and IV-severe). The staging is based on location, extent, and depth of endometriosis implants, the severity of adhesions and severity of ovarian endometriomas. In current study, we evaluate the association of *Fas* and *FasL* polymorphisms and stages of endometriosis in Iranian people.
Materials and Methods: As case group 112 women with confirmed endometriosis and as controls 110 women who had no evidence of endometriosis were selected. *Fas* -670 A/G and *FasL* -844 C/T polymorphisms were evaluated in extracted DNA samples using PCR-RFLP. The relation of gene variation and endometriosis stages was evaluated.
Results: The most endometriosis patients were in stage III (33%). The genotypes of *Fas* -670A/G and *FasL* -844C/T were not associated with the endometriosis stages (p=0.625 and p=0.231 respectively). Most of the patients with -670 AA genotype were in stage III and IV of endometriosis (29.5% in both stages). However, most of the patients with -670 AG genotype were in stage II of the disease (39.1%). While 40.9% of patients with -670 GG genotype reported to have stage III of endometriosis. Regarding -844 CC genotype, 36.2% of cases had stage II, whereas 43.8% of -844 CT and 36.4% of -844 TT genotypes were presented with stage II and IV of endometriosis.
Conclusion: We found no significant evidence for the association between *Fas* and *FasL* gene polymorphisms and the stages of endometriosis.
Key words: *Endometriosis stages, Fas, FasL, Polymorphism.*

P-94
The rates of protamine 1 and 2 gene expression of sperm chromatin in varicocele patients

Nayeri M[1], Talebi AR[2], Seifati SM[1].
1. *Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

Email: prof_talebi@ssu.ac.ir

Introduction: Varicocele or disturbance of testicular blood circulation is considered of one of the main cause of male infertility with decrease of sperm fertility potential. Although, there are many studies on the effects of varicocele on sperm parameters and some functional capabilities of these cells, but there are few data on sperm chromatin molecular changes in these patients. So, the aim of present study is the evaluation of sperm chromatin/DNA integrity and the changes of protamine (1 and 2) genes expression in varicoceles.
Materials and Methods: In total 25 sperm samples as patient group and 25 sperm samples as control group were evaluated at first for sperm parameters according to WHO criteria. For sperm chromatin and DNA integrity, the CMA3 staining and TUNEL assay were done respectively. To examine the sperm protamine (1 and 2) expression, after RNA extraction and prepairing of cDNA, the levels of protamines mRNAs were determined by RT-PCR method.
Results: The results showed that the sperm parameters significantly decreased in varicocele patients when compared to controls. In sperm chromatin condensation, the percentage of spermatozoa with protamine deficiency was greater than control ones. In the results of TUNEL assay, the rate of apoptotic spermatozoa showed significantly increase in comparison with control fertile men. In molecular evaluations, we showed that the level of protamine 1 expression decreased in patients group, but the level of protamine 2 expressions didn't show any significant difference with control group. It should be noted that the ratio of protamines was changed to about 1/2 of the ratio in control group.
Conclusion: In conclusion, our results showed that in the case of varicocele, not only we have a reduction in sperm parameters, the rates of sperm chromatin

condensation will decrease and sperm apoptosis will increase. On the other hand, the level of sperm protamine expression changes and so we can use this, as a good marker of male infertility due to the varicocele.

Key words: *Varicocele, Sperm, Chromatin, Protamine expression.*

P-95

Pregnancy outcome of "delayed start" GnRH antagonist protocol versus GnRH antagonist protocol in poor responders. Clinical trial study

Hosseinisadat R.
Department of Obstetrics and Gynecology, Kerman University of Medical Sciences, Kerman, Iran.
Email: robabehosseinisadat@yahoo.com

Introduction: Management of poor-responding patients is still major challenge in assisted reproductive techniques (ART). Delayed-start GnRH antagonist protocol is one of the treatment method that recommend to these patients, but study about this protocol is inadequate.

Materials and Methods: This study was a randomized clinical trial. 60 infertile women with Bologna criteria for ovarian poor responders who were candidate for in vitro fertilization (IVF) were participated in two groups. In case group (n=30) Delayed-start GnRH antagonist protocol administered estrogen priming followed by early follicular-phase GnRH antagonist treatment for 7 days before ovarian stimulation with gonadotropin. Control group (n=30) treated with estrogen priming antagonist protocol. Finally mature oocytes number, embryo number and pregnancy rate compared in two groups.

Results: Chemical, clinical, ongoing pregnancy and implantation rate in delayed-start cycles was higher although was not statistically significant. Endometrial thickness was significantly higher in case group. There were not statistically significant differences in total number and mature oocyte, two pronuclei, embryo and IVF outcomes in two groups.

Conclusion: Delayed-start GnRH antagonist protocol can be a new method for treatment of poor ovarian responders.

Key words: *Pregnancy outcome, Poor responder, In vitro fertilization, GnRH antagonist protocol.*

P-96

Th17 cells and related cytokines in unexplained recurrent spontaneous miscarriage at the implantation window

Saifi B[1], Rezaee SAR[2], Mehdizadeh M[3].
1. *Department of Anatomical Sciences, Mashhad Medical Science Branch, Islamic Azad University, Mashhad, Iran.*
2. *Inflammation and Inflammatory Diseases Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *Cellular and Molecular Research Center, Faculty of Advanced Technology in Medicine, Anatomy Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Email: bita_saifi@yahoo.com

Introduction: Unexplained recurrent spontaneous abortion (RSA) might be caused by the mother's immunological rejection of the fetus. In this cross-sectional study, the percentage of T helper 17 (Th17), T regulatory (Treg) cells and their cytokines as the main players of immunomodulation in peripheral blood lymphocytes during the luteal phase of 20 women with unexplained RSA were compared with 20 normal non-pregnant women.

Materials and Methods: In this cross-sectional study, the percentage of T helper 17 (Th17), T regulatory (Treg) cells and their cytokines as the main players of immunomodulation in peripheral blood lymphocytes during the luteal phase of 20 women with unexplained RSA were compared with 20 normal non-pregnant women.

Results: The percentage of Treg cells in the former was significantly lower compared with controls. The percentage of Th17 cells in the former was higher than controls. Expression of IL-23, IL-17, IL-6 cytokines in the former was significantly higher than controls, but the higher expression of IL-21 was not significant. The gene expression of TGF-β and FoxP3 in the former was lower than controls. Significant positive correlations were found between the percentage of Th17 cells with IL-23, IL-6 and IL-17 and between expression of IL-23 and IL-6 and IL-17. IL-6 gene expression showed a significant positive correlation with IL-17.

Conclusion: Imbalance of Th17–Treg cells and the consequent changes in cytokine expression might be implicated in the pathogenesis of unexplained RSA and may provide new insight into the immunoregulatory events at the maternal-fetal interface.

Key words: *IL-17, IL-21, IL-23, IL-6, TGF-β, Unexplained recurrent spontaneous miscarriage.*

P-97

Association of Toll-Like Receptor 3, 5 with susceptibility to tubal pregnancy

Chekini Z[1], Ramazanali F[1], Amirchaghmaghi E[1, 2], Gholamnezhadjafari R[3], Saboori Darabi S[1], Zarezade N[1], Taheri Barayjani R[1], Azadi R[1], Moini A[1, 4], Aflatoonian R[1].
1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR,Tehran, Iran.*
3. *Department of Immunology, School of Medicine, Isfahan Universty of Medical Sciences, Isfahan, Iran.*
4. *Obstetrics and Gynecology Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Email: a_moini@royaninstitute.org; R.Aflatoonian@gmail.com

Introduction: Ectopic Pregnancy (EP) is an abnormal pregnancy that embryo implants outside the uterus and most often in the Fallopian Tube (FT). Infection and pelvic disorders including pelvic inflammatory disease (PID) can result in adverse reproductive outcomes such as ectopic pregnancy. Dysfunction of innate immune system plays a critical role in pathogenesis of EP. Toll-like receptors (TLRs) have considerable performance in early host defense against invading pathogens.
Materials and Methods: Ten control women who underwent hysterectomy surgery were participant in this case-control study. They received human chorionic gonadotropin (hCG) in 14 days leading up to hysterectomy to produce a condition of pseudo-pregnancy. In ectopic pregnancy group, 10 women were included. The age range of control and EP groups were 26-40 and 32-46 years, respectively. Biopsies from infundibulum, ampulla and isthmus of FT were obtained in both groups. RT-PCR was used to show the existence of TLR3,5 expression in FT. Quantitative survey of TLR3,5 gene expression was assessed by using Q-PCR. Comparison between groups was by analysis of mean±SEM.
Results: Using RT-PCR the presence of antiviral TLR3 and TLR5 genes have been shown isthmus, ampulla and infandibulum of the FT in both groups. Q-PCR has confirmed relative TLR3 expression in all regions of FT carrying EP is lower than FT in pseudo pregnant women. Also, lower expression of TLR5 was detected in all region of FTs from case compared with control. This difference was statistically significant ($p \leq 0.05$).
Conclusion: The lower expression of TLR3,5 in difference regions of fallopian tube from EP women and increasing of infection following TLRs decline leads to ectopic implantation of embryo.
Key words: *Ectopic pregnancy, Fallopian tube, Innate immunity, TLR3, TLR5.*

P-98
Innate immunity response via Toll-Like Receptor 2,4 in tubal pregnancy

Chekini Z[1], Ramazanali F[1], Amirchaghmaghi E[1, 2], Gholamnezhadjafari R[3], Azadi R[1], Taheri Barayjani R[1], Zarezade N[1], Saboori Darabi S[1], Moini A[1, 4], Aflatoonian R[1].

1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR,Tehran, Iran.*
3. *Department of Immunology, School of Medicine, Isfahan Universty of Medical Sciences, Isfahan, Iran.*
4. *Obstetrics and Gynecology Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Email: a_moini@royaninstitute.org; R.Aflatoonian@gmail.com

Introduction: The innate immune system is the first line of defense against pathogens. Toll like receptors (TLRs) have a key role in mediating of innate immune system and immunological events on female genital tract and pregnancy. Ectopic Pregnancy (EP) is a pregnancy where the fertilized ovum implants outside the uterus and more than 98% of them are located in the Fallopian tube (FT). Ch. trachomatis infection can causes tubal EP that initiate innate immune responses by ligating members of the TLRs family of pattern recognition receptors, in particular TLR2, 4.
Materials and Methods: In this case-control study, biopsies from infundibulum, ampulla and isthmus of FT were obtained from 10 women (26-40 yr) who underwent salpingectomy for EP. In addition, human chorionic gonadotropin (hCG) was injected in 14 days leading up to hysterectomy to produce a state of pseudo-pregnancy as a control group (32-46 yr). In this investigation TLR2, 4 expressions was survey with RT-PCR. Also Q-PCR was used to compare quantitative expression of TLR2, 4 between two groups.
Results: Using RT-PCR shown that TLR4 was expressed in three regions of control group. In case group, TLR4 was not expressed in infundibulum and ampulla. In addition, TLR2 expression has found in infundibulum, ampulla and isthmus from case and control group. Using Q-PCR shown that, in all regions of case group TLR2, 4 expression levels were significantly lower than control group ($p \leq 0.05$).
Conclusion: TLRs protected against invading microorganisms in the female reproductive tract and mediated several interactions between immune and reproductive system. It seems reduce expression of TLR2,4 leading to the dysregulation of factors involved in implantation and ciliary beat within FT resulted increase risk of EP.
Key words: *Ectopic pregnancy, Fallopian tube, Innate immunity, TLR2, TLR4.*

P-99
Increased *VEGF* gene expression in patients with repeated implantation failure (RIF) following endometrial injury

Aghajanpour S[1], Amirchaghmaghi E[1], Yahyaei A[1], Ashrafi M[1, 2], Aflatoonian R[1].

1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Obstetrics and Gynaecology, Faculty of Medicine, Iran University of Medical Science, Tehran, Iran.*

Email: R.Aflatoonian@gmail.com

Introduction: Embryo implantation is a complex process that is necessary for successful pregnancy. Implantation failure can occur due to several factors. It is assumed that an absent receptive endometrium is the main cause of implantation failure. Many strategies have been done to improve the implantation rate in IVF/ICSI cycles. Endometrial injury is one of these

procedures which recently obtained more popularity. It seems injury can trigger angiogenesis which is an important aspect of successful implantation. In the present study, we investigated whether endometrial injury during proliferative phase of menstrual cycle before embryo transfer can improve *VEGF* (vascular endothelial growth factor) gene expression (as a pivotal marker of uterine angiogenesis) in compare to patients in the control group.

Materials and Methods: In this randomized controlled trial (RCT) study, twenty women with repeated implantation failure (RIF) who failed to conceive during two or more IVF/ICSI cycles and embryo transfer (ET), randomly divided into two study groups (N=10 in both case and control group). Samples were obtained following endometrial injury on day 9 of the preceding menstrual cycle in case group. Pipelle endometrial sampling was done twice: One in the follicular phase and again in the luteal phase but it was done once in the control group just in the luteal phase for genomic evaluation. RNA extraction and cDNA synthesis from endometrial biopsy were performed. *VEGF* gene expression was investigated by quantitative real-time PCR.

Results: *VEGF* gene expression was detected in endometrial samples of both groups. The mean relative expression of *VEGF* gene was higher in case endometrial injury group compared to control group.

Conclusion: Evaluation of implantation markers such as *VEGF* may help to predict pregnancy outcome and detect occult implantation deficiency. In this study we showed that endometrial injury induces angiogenesis via increasing *VEGF* as a potent angiogenic factor responsible for vascular development.

Key words*: Endometrial injury, Vascular endothelial growth factor (VEGF), Repeated implantation failure (RIF).*

P-100

The majority of poor quality embryos that reach to blastocyst have normal chromosomal integrity

Taheri F[1], Khalili MA[1], Aflatoonian A[1], Kalantar SM[1], Agha-Rahimi A[1], Soleimani M[1, 2], Montazeri F[1, 3], Fesahat F[1].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
3. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: taherifatemeh86@yahoo.com

Introduction: In ART procedure, large numbers of surplus poor-quality embryos are formed with developmental potential. With efficient culture systems, it is more reliable to culture embryos to blastocyst stage in vitro. Morphological assessments are the main way to select embryos, and studies have considered relationship between embryo morphology and aneuploidy, but this relationship is not absolute.

Materials and Methods: Only low quality cleavage embryos that were not suitable for transfer or cryopreservation met the inclusion criteria. Multinucleated embryos and the embryos generated from woman >35 yr old were excluded. The embryos obtained from ejaculated spermatozoa were included, and degenerated or arrested embryos were excluded from the study. The embryos were cultured in optimal condition and after reaching to blastocyst stage, they were prepared for PGD by FISH technique.

Results: Out of 20 blastocysts resulting from culture of surplus poor-quality embryos, 14 (70%) blastocysts were normal for X, Y, 13, 18 and 21 chromosomes. However the rest of embryos (n=6) were diagnosed as turner mosaism, turner, monosomy 18, monosomy 21, trisomy 13 and trisomy 18.

Conclusion: Morphological assessment alone does not guarantee the chromosomal normality of the poor-quality embryos that have reached to blastocyst. However, data showed that majority of poor quality embryos can develop further and repeat of IVF cycles can be prevented.

Key words: *Poor quality embryo, Chromosomal abnormality, Blastocyst, FISH.*

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P-101

Allelic frequency of peroxisome proliferator activated receptor gamma Pro12Ala polymorphism in Iranian patients with polycystic ovary syndrome

Jafari F[1], Kalantar SM[2], Miresmaeili SM[1].

1. *Department of Biology, Science and Art University, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductivev Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fatemehjafari175@gmail.com

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting up to 7% of women of reproductive age. The syndrome is characterized by oligo-anovulation, hyper androgenism and polycystic ovaries. Polycystic ovary syndrome is a metabolic disorder resulting from the interaction of genetic predisposition and environmental risk factors. The nuclear hormone receptor peroxisome proliferator activated receptor gamma (PPARγ) is an important transcription factor regulating adipocyte differentiation, lipid and glucose homeostasis, and insulin sensitivity. The Pro12Ala polymorphism of *PPAR* gamma gene has been associated with reduced transcriptional activity of PPAR gamma and presence of Ala isoform has been

linked to higher insulin sensitivity and lower body mass index.

Materials and Methods: A total of 100 reproductive-aged women were included in this case-control study were diagnosed as a PCOS based on Rotterdam criteria and 100 healthy women with no evidence of PCOS were recruited as controls. The case and control group genotyped using the technique PCR-RFLP for Pro12Ala polymorphism.

Results: The CC allele frequency was 67% in patients group. Among studied subjects 2% were abnormal homozygous and 31% were genotyped as heterozygous. The allele frequency differences between groups were estimated using Chi-squar test, and we have seen significance difference ($p \leq 0.0001$) between two groups. Also, FSH and LH levels were difference in patients and control groups.

Conclusion: This study demonstrate difference in allelic distribution in Iranian population. Also, the allele frequency of Iranian population is similar to Indian population. Future association studies are required to reveal clinical consequence of for Pro12Ala polymorphism in carrier individuals.

Key words: *PCOS, PPARγ, Polymorphism, PCR-RFLP, Genotyping, Polycystic ovary syndrome.*

P-102

Association of A222V polymorphism in *MTHFR* gene with male infertility: a meta-analysis in Iranian population

Karimian M[1], Ghavampoor F[2].

1. *School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.*
2. *School of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: mdkarimian@gmail.com

Introduction: Male infertility is a main issue for juvenile couples. About 50% of infertility causes refer to male factors. Genetic factors such as genetic polymorphisms in key genes could affect male infertility. One of key gene polymorphisms is *MTHFR*-A222V which may influence male infertility. There are three case-control study about the association of A222V transition with male infertility in Iranian population, but the results are approximately inconclusive.

Materials and Methods: We found the eligible studies by search in suitable databases. Then, the data was extracted from included studies and was analyzed by Open Meta [analyst] Software.

Results: Our data revealed that there is a significant association of *MTHFR*-A222V with male infertility in TT vs. CC (OR=2.197, 95% CI=1.431-3.372, $p \leq 0.001$, Pheterogeneity= 0.526, I2=0%) and CT vs. CC (OR=1.472, 95% CI=1.156-1.875, p=0.002, Pheterogeneity=0.767, I2=0%) models within Iranian population.

Conclusion: According to our results, *MTHFR*-A222V may be a strong biomarker for screening of susceptible infertile men in Iranian population.

Key words: *Male infertility, MTHFR gene, Genetic polymorphism, Meta-analysis.*

P-103

Studying the impact of *MTHFR* gene mutants on recurrent abortion

Fazel Tolami H[1], Mehrafza M[2], Askari M[1].

1. *Department of Genetic, Mehr Medical Institute, Rasht, Iran.*
2. *Department of Obstetrics and Gynaecology, Mehr Medical Institute, Rasht, Iran.*

Email: hf.tolami@gmail.com

Introduction: Spontaneous recurrent abortion occurs in 15-20% of the pregnancies which have been confirmed by clinical tests. failing in pregnancy and recurrent abortion, impose economic and mental costs on families and the society. it is caused by various and sometimes unknown reasons. this problem is partly resulted from Thrombophilia disorders in patients which maybe either acquired or inherited .in terms of inheritance ,one of the important gene is methylene tetrahydrofolate reductase (*MTHFR*).

Materials and Methods: This study have been implemented on 65 women having a history of 2 or more pregnancy loss in the first trimester of their pregnancy and on the other hand, 56 women as the control group having at least one child and no history of spontaneous pregnancy loss. after investigating the medical file of the patients and genetic counseling , the sampling was performed and 2 gene polymorphisms (A1298C and C776T) were examined by PCR-RFLP technique.

Results: From 65 women experienced recurrent abortion, 21 (34.4%) were heterozygote in *MTHFR* gene, including 4 cases in both C667T and A1298C, 10 cases in C667T and 7 in A1298C, while the results in control group were 0, 6 and 3 respectively.

Conclusion: Our results indicated that both *MTHFR* polymorphisms could be related to recurrent abortion in women.

Key words: *Recurrent abortion, MTHFR, Thrombophilia, Polymorphism.*

P-104

Micro-RNAs and their roles in breast cancer pathogenesis; An updated review article

Bakhshi Aliabad MH[1], Kheirandish Sh[1], Zarezade Z[1], Saeidi N[1], Kabiri H[1], Khazaei Z[1], Sahamifar H[1],Taghvaei F[1], Jafarzadeh Rastin S[1], Zare M[1], Bahrami-Babaheidar S[1], Mazaheri M[2].

1. *Department of Human Genetics, International Campus, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
2. *Department of Genetics, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: bakhshi_mohammad2002@yahoo.com

Introduction: Micro-RNAs (miRNAs) were discovered in 1993 by Rosalind Lee and Rhonda Feinbaum while

working on the lin-14 gene in the C. Elegans. In fact, one of the most important recent advances in biochemical research was the discovery of noncoding long 22 nucleotide RNAs called miRNAs that are involved in regulating genes' expressions via mRNA degradation or preventing its translation and modulating a variety of basic cellular processes. Today a few number of miRNAs have been studied and the performance of this number included cell differentiation, proliferation, apoptosis, and anti-viral and anti-cancer defenses. About 50% of miRNAs are placed within genes and it is expected that approximately 50% of them be placed in introns. More than half of miRNA genes are located on chromosome's fragile sites that allow duplication, deletion, and cell movement during the cancer development process. These areas can affect the expression of miRNAs. Recent research has shown that these molecules can act as either oncogenes or tumor suppressors. Given the importance of etiology and treatment of tumors and due to many unknown causes of cancers, extensive studies have discussed around the role of these micro molecules in the pathogenesis of cancers. Many studies have examined the possible molecules involved in cancer. Today, several review articles have been published regarding several aspects of miRNAs in breast cancer. In this review article, we attempted to collate recent articles regarding miRNAs in breast cancer to update our knowledge and discuses about the production and performance of miRNAs and their involvement in the pathogenesis of breast cancer.

Materials and Methods: In order to find recent investigations regarding the roles of miRNAs in breast cancer, three main databases including PubMed, Scopus, and Google Scholar were searched using "miRNA, micro-RNA, and breast cancer" keywords.

Results: According to the results achieved in the presented studies, it seems that miRNAs b27, 31, 125, 141, 145, a2196, 200 family, 205, 206, 210, 429, and 499 along with let-7 are the molecules that have pre-apoptotic function and reduce the expression of oncogenic proteins and increase the expression of anti-apoptotic molecules. Accordingly, it may be concluded that the prevention of cancers such as breast cancer is plausible by increasing their expression.

Conclusion: Given the role of miRNAs in cancer induction or prevention as well as invasiveness or non-invasiveness, it seems that these micro molecules can be used as biomarkers for early detection of breast cancer and progression. Release of miRNAs from the tumor to the blood circulation is performed through the exosome vesicles and apoptotic bodies, hence, miRNAs present in blood circulation can be used as diagnostic indicators. Therefore, the levels of miRNAs' expression can be considered in the monitoring of tumor status. It is worth to note that the removal of primary tumor reduces the rotational miRNAs. Hence, this investigation showed that we can consider these micro molecules in the identification of patients' status and treatment pathways.

Key words: *Breast cancer, Micro-RNA, Pathogenesis.*

P-105

Investigating the association between estrogen receptor beta gene +1730 G/A polymorphism and risk of PCOs

Karimi R[1, 2], Montazeri F[2], Sheikhha MH[3], Kalantar SM[2], Dehghani MR[2], Karimi F[3], Golzade M[1, 2].

1. *Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: roghayehkarimi21@yahoo.com

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, with a prevalence that varies from 4-7%. The etiology of PCOS is yet to be elucidated, but several studies have been suggested role of genetic factors in its pathogenesis.

Materials and Methods: DNA extraction and ARMS-PCR were used to detect the polymorphic genotypes. Chi-square test and the frequency differences of alleles and genotypes between two groups were compared.

Results: Results obtained from 50 PCOS and 50 control samples showed a significant difference in the genotype distribution (non-GG rates were 12.5% for patients with PCOS and 26.6% for controls).

Conclusion: Considering the significant association of this polymorphism; for confirming this association, study continues on a larger number of PCOS and control samples of women referred to Research and Clinical Center for Infertility, Yazd, Iran.

Key words: *Estrogen receptor b, Gene, Polycystic ovary syndrome, 1730 G/A polymorphism.*

P-106

Association of rs1264457 genetics variants of *HLA-E* with unexplained recurrent pregnancy loss

Zarezade Z, Ashrafzade Mehrjardi HR, Ghasemi N.

Recurent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: zarezadezz@gmail.com

Introduction: Recurrent pregnancy loss (RPL) is defined by two or more failed pregnancies and approximately 1.5% of women are involve with this clinical problem. Human Leukocyte Antigen (HLA)-E is involve in reprogramming immune responses at fetal-maternal interface during pregnancy. This study evaluated the rs1264457 variants of *HLA-E* in women with unexplained recurrent pregnancy loss (uRPL), with 2 or more pregnancy loss and women with a history of successful pregnancy.

Materials and Methods: In this case-control study including 194 unexplained RPL couples and healthy women with a history of successful pregnancy that referred to Yazd Infertility Center from 07/2014 to 08/2016, rs1264457 genetics variants polymorphism was implemented using restriction fragment length polymorphism technique (PCR-RFLP) and gel electrophoresis.
Results: In this case-control study, the difference in allele frequencies between cases and control was significant.
Conclusion: Hence, evidence was provided for association of rs1264457 polymorphism with RPL, further studies are required to confirm our results.
Key words: *rs1264457 genetics variants, HLA-E, Unexplained recurrent pregnancy loss, Polymorphism.*

P-107
Expression of miRNA-15, miRNA-16 and miRNA-21 in apoptotic pathway in ovarian cancer patients

Zarezade Z[1], Kheirandish Sh[1], Saeidi N[1], Zaimy MA[2], Sheikhha MH[3], Ghasemi N[4], Kalantar SM[3].
1. *Department of Genetic, Shahid Sadoughi Univeresity of Medical Sciences, Yazd, Iran.*
2. *Department of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi Univeresity of Medical Sciences, Yazd, Iran.*
4. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi Univeresity of Medical Sciences, Yazd, Iran.*

Email: zarezadezz@gmail.com

Introduction: Ovarian cancer has the highest mortality rate among women-specific cancers, because more than 65 -75% of women with ovarian cancer are diagnosed only in advanced stages (III and IV) of disease. Therefore, screening strategies for early diagnosis this disease are very important. MiRNAs belong to a class of small noncoding RNAs containing 19-25 nucleotides that are not protein coding and can breakdown or prevent translation of mRNA by binding to the 3' UTR of it in the post-transcriptional stage. MiRNAs plays critical roles in biological processes during tumorigenesis.
Materials and Methods: In this study, serum samples were collected from patients with stage III or IV epithelial ovarian cancer referred to Shahid Sadoughi Hospital from 07/2015 to 03/2016. The controls were among people referred to Yazd Blood Transfusion Center with female gender, similar average age and without any history of ovarian cancer or drug use. In order to evaluate miRNAs expression alteration, RNA was extracted from serum of ovarian cancer patients and controls. After reverse transcription, real-time qPCR was performed and data was analyzed using Rest-2009 and Graph Pad software.
Results: Our data revealed that two miRNAs (miR-15, miR-16) were down-regulated,but miR-21 was upregulated in serum of ovarian cancer patients compared with control.
Conclusion: This study demonstrated three microRNAs in apoptosis pathway were differently expressed in serum samples versus normal serum. Downregulation of these two miRNAs (miR-15, miR-16) indicates their possible tumor suppressing activity and upregulation of miR-21shows their possible oncogenic roles. According to these results, miR-15,miR-16 and miR-21 could be used as ovarian cancer diagnostic markers.
Key words: *Ovarian cancer, Apoptosis pathway, miR-15, miR-16, miR-21, Real time PCR*

P-108
The role of *ERβ* polymorphism on ovarian response in IVF cycle

Parsa E[1], Sheikhha MH[2], Hoseini SM[3], Nemayandeh SM[4], Akhavan Sales Zh[5].
1. *Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Yazd, Iran.*
2. *Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Cell and Molecular Biology Department, Ferdowsi University, Mashhad, Iran.*
4. *Yazd Cardiovascular Research Center, Shahid Sadooghi University of Medical Sciences, Yazd, Iran.*
5. *Department of Immunology, Shahid Sadooghi University of Medical Sciences, International Campus,Yazd, Iran.*

Email: elhamparsa@live.com

Introduction: Infertility is defined as one-year unprotected intercourse without pregnancy. Estrogen is also known to be involved in many pathological processes.
Materials and Methods: A cross-sectional study was performed involving 91 infertile women and the relationship between genotype distribution of the +1730 G/A polymorphism in the *ERβ* gene and the mean number of follicles and oocytes, their mean ratio, mean number of embryos, mean size of the follicles and pregnancy rates was measured. The *ERβ* gene +1730 G/A polymorphism were identified by the amplification-refractory mutation system (ARMS-PCR).
Results: Genotypes GG, GA and AA of the *ERβ* gene presented frequencies of 27.5%, 67% and 5.5%, respectively, in the infertile women. The results of study showed that the mean number of follicles and oocytes, their mean ratio, mean number of embryos, mean size of the follicles and pregnancy rates have not been related to different genotypes.
Conclusion: According to the endocrine and paracrine factors which are involved in the ovulation induction and maturation of oocytes, further studies are required to find out other gene polymorphisms affecting estrogen receptor efficacy in the infertile women.
Key words: *Gene polymorphism, Estrogen receptor gene, Infertility.*

P-109

Comparison of immunohistochemistry (IHC) ad fluorescence in-situ hybridization (FISH) assay for assessment of *HER-2/neu* gene amplication in breast cancer specimens

Karimi F[1], Montazeri F[2], Kalantar SM[3], Zarrabi N[4], Taghipour Sh[1], Karimi R[2], Sabbagh-Nejad Yazd S[2].

1. *Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.*

Email: fayeghehkarimi@yahoo.com

Introduction: Accurate assessment of *HER-2/neu* gene status in breast cancer is important for management of the disease. Several studies have been done to compare the results of these methods to arrive at a "gold standard" for the HER2 status testing, the existing literature is still unclear about the most ideal and specific test for determination of the HER2 status.
Materials and Methods: In this study we compared FISH and immunohistochemistry (IHC) for the investigation of *HER-2/neu* gene amplification. 25 formalin fixed paraffin embedded breast cancer tissues were analysed using these methods. A large number of samples are understudy.
Results: IHC reports were available for all of these samples. Of the 19 patients with the score of 2+ by IHC, all samples were FISH positive for *HER-2/neu* gene amplification. 2 patients showed equivocal results by FISH because 2 of 3 sample with the score of 3+ by IHC showed FISH negative result. Our results indicated that HER-2/neu status by FISH should be performed in all cases of breast tumour with a 2+/ 3+ score by IHC.
Conclusion: Cases demonstrating a 3+ score by IHC may be subjected to FISH to rule out polysomy of chromosome 17 which could be falsely interpreted as HER-2/neu overexpression by IHC analysis. There is also a need for establishing a clinically validated cut-off value for HER-2/neu FISH amplification against IHC which may be further compared and calibrated.
Key words: *Breast cancer, HER2/neu gene amplification, FISH, IHC.*

P-110

The karyotype analysis of Iranian infertile men with cryptorchidism

Sharifi N[1, 2], Mohseni Meybodi A[2], Sabbaghian M[3], Farrahi F[3], Almadani N[2].

1. *Department of Molecular Genetic, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: neda_sh2111@yahoo.com

Introduction: Testicular descent is a main part of normal sexual development in boys. This sensitive process has two phases. It is sometimes insufficient and results in cryptorchidism or undescended testis (UDT). It has different reasons like environmental, endocrine or genetic factors. It is proved that cryptorchidism can be a main risk factor for infertility and testicular cancer. So it is an important disease due to infertility consequence. Many studies have been reported about cryptorchid boys with chromosomal anomalies as case report.
Materials and Methods: This research was conducted on 522 patients with undescended testis who referred to Royan institute during five years. They were selected after clinical examinations, hormonal tests, and semen and karyotype analysis. All the patients were divided into azoosperm or oligoosperm, unilateral or bilateral group to survey their cytogenetic data. Karyotype analysis was performed using standard GTG banding technique.
Results: From all patients, 348 azoosperm (66.66%) and 174 oligoosperm (33.4%) were detected. The chromosomal alterations were diagnosed in 45 cases (8.62%). Among these, seven had normal variations (1.34%) and the remaining 38 patients (7.3%) with abnormal karyotypes that 30 of them had unilateral and just eight cases had bilateral cryptorchidism. The most common abnormalities were Klinefelter syndrome and mosaic patterns, in 18 (3.44%) and 10 cases (1.91%) respectively. Also sex reversal, structural and 47, XYY syndrome were detected with lower incidence.
Conclusion: It seems that cryptorchidism is associated with abnormal karyotype. In case of using assisted reproduction technology for infertility treatments, it is recommended to have a karyotype analysis in patients with cryptorchidism because they may have chromosomal abnormalities.
Key words: *Cryptorchidism, Undescended testis, Karyotype, Infertility.*

P-111

Assessment of genetic variation in exon 10 of *DPY19L2* in total and partial globozoospermic patients

Alimohammadi F[1], Sabbaghian M[2], Hashemi M[3], Totonchi M[3].

1. *Department of Genetic, Tehran Medical Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3. *Department of Genetic, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: Fatemeh_alimohammadi@yahoo.com

Introduction: Globozoospermia is a rare but severe disorder causing male infertility, which characterized by

round-headed spermatozoa devoid of an acrosome. The pathogenesis of globozoospermia most probably originates in spermiogenesis, more specifically in acrosome formation and sperm head elongation. Globozoospermia is mainly due to *DPY19L2* deletion via non-allelic homologous recombination between the flanking low copy repeats. The aim of our study was to assess exon 10 in patients with total and partial globozoospermia.

Materials and Methods: In this study we performed a case-control study of 63 infertile men with total and partial globozoospermia and 41 normozoospermic men as control group. DNA samples were extracted from peripheral blood using salting out method. In first step, we screened for the deletion of *DPY19L2* in all patients. In second step, exon 10 was studied in non-deleted patients. Exon 10 was amplified by PCR and products were analysed by sequencing to determine genetic changes of the mentioned area.

Results: The results showed a whole *DPYI9L2* gene deletion in 22 of 63 patients with total globozoospermia. However, none of the 34 partial globozoospermic patients showed this deletion. PCR product sequencing results illustrated three single nucleotide polymorphism in the intronic regions with (rs61936086, T>C and rs61936087, A>G) in 19 patients and 11 control and (rs61936086, T>C) in 11 patients and 22 control. Except rs61936086 and rs61936087 statistically significant association was shown in rs61936086 between two groups (p=0.02).

Conclusion: The results showed homozygous deletion of the *DPY19L2* gene in 45% of globozoospermic patients. Our result suggest that these SNPs (rs61936086, rs61936087, rs61936087) seems to be normal variants in our population of study, it can be concluded that this location has no effect on globozoospermia in Iranian infertile men. *DPYI9L2* is composed of 22 exons so, evaluation of other exons of this gene is recommended.

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

P-112

Effects of plastic materials on health and reproductive axis of women at reproductive age

Simony E, Mirahi A
School of Nursing and Midwifery, Islamic Azad University, Tehra Branch, Tehran, Iran.
Email: unatan.simony@yahoo.com

Introduction: Estrogen-like endocrine disrupting chemicals (EEDC) are exogenous, man-made chemicals that alter the functions of the endocrine system. EEDC are found abundantly in the environment are on residential buildings, cars, furniture, plastics, products such as baby feeding bottles, lining in tin-food containers and even in children's toys. The striking similarity in structure of EEDC molecules and estrogen have been traced to influence our endogenous hormone balance, and cause various health defects relating to female reproductive disorders like: breast cancer, disorders of the ovary and uterus, fetal growth restriction, and pregnancy.

Materials and Methods: To achieve this aim, the related articles from accredited databases such as: Cochran, Medline, Google Scholar, PubMed, Embrace and Sid were investigated. The materials relating to Plastic materials and women's health were studied then, content analysis was carried out and the results were summed up.

Results: EEDC affect the synthesis, metabolism, binding, transport or any other cellular responses of natural estrogens. They alter the functions of estrogen by primarily interfering the binding with the hormone's receptor and thereby causing an impact on a wide range of signaling processes. Suggested that Xenoestrogens are required about 1000 to 10,000 fold higher concentration in order to show a similar effect through ER mediated gene regulation. Estrogen exerts their effects on cells mainly via two nuclear receptors, estrogen receptor a (ERa) and estrogen receptor b (ERb). BPA (Bisphenol A) is the monomer, and the most commonly found EEDC in the environment. It Is used to make polycarbonate plastics and epoxy resins, which is used to make a variety of household products used daily. Among these products are reusable plastic containers like tupperware, baby formula bottles, dental sealants used in the manufacture of polycarbonate plastics and epoxy resins, exposure can occur via plastic food containers (especially when heated or microwaved), food and drink cans, baby bottles, and carbonless paper. A common complication of estrogen-like chemicals, especially in the face of these materials in prenatal and pre-puberty, puberty and after puberty, are precocious puberty, polycystic ovarian syndrome, amenorrhea, endometriosis, uterus myoma, obesity and increase in breast cancer in women.

Conclusion: Increased consumption of plastic containers and disposable food storage among Iranian families and grocery stores, restaurants etc. also increase the consumption of mineral water bottles that we see so prevalent today, perhaps is an important and effective factor in increasing women's disorders such as polycystic ovarian syndrome, uterine myoma, menstrual disorders, endometriosis, infertility and ultimately increase women's cancers such as: ovarian, breast, and uterus. So, increasing the number of researches, education and rising awareness of therapists, all people, especially girls in puberty, in this field can help us to achieve the goals such as: increase fertility, women's health and society through reduce costs and Ccomplications of assisted reproductive techniques.

Key words: *Plastic materials, Women's health, Reproductive.*

P-113

Studying the chromosomal abnormalities in infertile men from Northern Iran

Askari M[1], Mehrafza M[2], Fazel Tolami H[1].
1. *Department of Genetic, Mehr Medical Institute, Rasht, Iran.*
2. *Department of Obstetrics and Gynaecology, Mehr Medical Institute, Rasht, Iran.*

Email: mgenetic.rasht@gmail.com

Introduction: Infertility refers to inability to conceive after having a year of unprotected sexual connection which has troubled 10-15% of the couples. The source of this problem in half of the cases is the male partner and the other half is caused by women related issues. Various causes affect the male fecundity among which the genetic factors such as structural and numerical disorders in chromosomes, Yq microdeletions and mutation in some of the specific genes, play important role in male sterility. According to the performed studies, the incidence of chromosomal abnormalities among the infertile men is 2-8%.
Materials and Methods: This research involved 101 male cases with a history of 2-25 yr of infertility who were recognized as azoospermic or oligospermic based on SFA test and referred to Mehr Infertility Clinic of Rasht. In this research the cases were followed through a process started from urological and genetic counseling and the necessity confirmation for performing the Karyotype test, preceded with sampling from the peripheral blood and Lymphocyte proliferation and finally the high resolution banding technique was utilized.
Results: The results of this study revealed the chromosomal abnormality in about 10% of the cases and most of them were related to Klinefelter syndrome. Also some cases of mosaic Klinefelter syndrome, reciprocal translocation, chromosome breakage, and acrocentric chromosome were observed in the patients. Moreover all the cases with sexual chromosome disorders were azoospermic.
Conclusion: Chromosomal abnormalities can be one of the main reasons of male infertility. However, fortunately it is detectable by a simple test and the result of the test would be important in following the relevant treatments, therefore it is suggested to be performed on infertile men.
Key words: *Infertile men, Chromosomal abnormality, Azoospermic, Oligospermic.*

P-114
The role of epigenetic modifications as a new diagnostic and therapeutic tools for improving male infertility

Vaseghi Dodaran H[1], Daghigh Kia H[1], Atshan M[2].
1. *Department of Animal Science, University of Tabriz, Tabriz, Iran.*
2. *Department of Basic Science and Advanced Technologies in Biology, University of Science and culture, Tehran, Iran.*

Email: h.vaseghi28@yahoo.com

Introduction: Male fertility is regulated by a number of genes, which regulate spermatogenesis and are required for sperm viability. The maintenance of genomic integrity is of vital importance for sperm cell. The pattern of gene expression in cells is different and induce alterations in functions of cells. Methylation, demethylation, acetylation, and deacetylation responsible for variable gene expression. Epigenetics has critical role in sperm development and function, fertilization, and post-fertilization events.
Materials and Methods: As a methodological approach, we considered to include all the articles retrieved from PubMed, Google scholar and SID search with the keywords "DNA Methylation", "Histone Modification" and "ncRNA"" combined with the key word "Epigenetic". The results were filtered by limiting the search to English manuscripts published within the last 5 yr that discussed studies of mammalian subjects.
Results: Several studies showed that Methylation of DNA plays an important role in the epigenetic control mechanism as genomic imprinting, the maintenance of genome stability and also gene expression regulation. Epigenetic modification at the N-terminal tail of histones plays significant role in both structural and functional states of the chromatin. Also, on other hand, in ART, unknown epigenetic mechanisms can affect life span of model animals.
Conclusion: Epigenetic reprogramming taking place throughout spermatogenesis is critical for correct development of spermatozoa. Therefore, the delineation of the epigenetic changes may be promising therapeutics for treating male infertility. Epigenetic modifications not only affect spermatogenesis but also affect disease susceptibility later in life. Sperm is epigenetically programmed to regulate gene transcription in embryos.
Key words: *Male infertility, Epigenetic, Methylation, Imprinting.*

P-115
Protective effect of Allium cepa (Onion) seeds (AC) extract on histopathology of testis in STZ-induced male rats

Haddad Kashani H.
Department of Gametogenesis, Kashan University of Medical Sciences, Kashan, Iran.
Email: hamedir2010@gmail.com

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. Allium cepa has been used throughout history as a medicinal drug. It has many compounds mostly containing sulfur, such as dialkyl disulfide (Alicin), diallyl disulfide (DAS), that are the cause of its antioxidant and protective properties.
Materials and Methods: Forty adult male Wistar rats (2 month old) were allocated into four groups of control, diabetic control, diabetic treated with 200 or 400 mg/kg/day of onion seed extract. Diabetes mellitus was induced using 60 mg/kg body weight of Streptozotocin as a single intraperitoneal injection. The extract was administered by stomach gavage for 28 days. The morphometric and histological structure of the testis, biochemical factors like glucose and testosterone levels were assessed. All analyses were done at the end of the four week study period. Data were compared by using

Kruskal Wallis Test, Dunnett T3 and the degree of significance was set at $p \leq 0.05$ and $p \leq 0.01$.

Results: In diabetic +200 rats, the numbers of primary spermatocytes were significantly increased. In diabetic +400 rats, Seminiferous Tubular Diameter was significantly increased and the level of testosterone hormone and testis weight was decreased significantly. In diabetic +200 and 400 rats, the numbers of spermatid, FBS and lumen diameter were significantly increased and the numbers of spermatozoa cells, body weight and volume density (VD) % lumen were decreased. Also, the numbers of spermatid in control diabetic rats was decreased.

Conclusion: Our finding indicated that onion seed extract might be useful as a supplementary protective agent against adverse effects of diabetes on reproductive system in diabetic men.

Key words: *Onion, Testis, Rat, Streptozotocin, Histopathology.*

P-116

Comparing the toxicity effect of dendrosomal curcumin with oxaliplatin in OVCAR3 ovarian cancer cell line

Seyed Hosseini E.
Department of Gametogenesis, Kashan University of Medical Sciences, Kashan, Iran.
elaheir.66@gmail.com

Introduction: Cancer is a hyperproliferative disorder that is usually treated by chemotherapeutic agents that are toxic not only to tumor cells but also to normal cells. In addition, these agents are highly expensive and thus not affordable for most. Traditional medicines are generally free of the deleterious side effects and usually inexpensive. Curcumin, a component of turmeric (Curcuma longa), is one such agent that is safe, affordable, and efficacious side effects, but its low bioavailability and water solubility represent the main disadvantages of its use. Here, to overcome these problems curcumin was encapsulated in a dendrosomal nanocarier and the effect of this compared with oxaliplatin a chemotherapy drug on proliferation of OVCAR3 ovarian cancer cell line.

Materials and Methods: OVCAR3 cell were cultured in RPMI1640 medium and treated with the dendrosomal curcumin and oxaliplatin at various concentrations for 24, 48 and 72 hr. Our study resulted in loss of cell viability were evaluated by MTT assay. Also mortality rate of encapsulated curcumin in nanocarrier of dendrosome was compared with normal curcumin by MTT tests.

Results: The results showed that the dendrosomal curcumin decreased cell viability in malignant cells as a concentration and time-dependent manner such as oxaliplatin. The IC50 values of dendrosomal curcumin and oxaliplatin against the OVCAR3 ovarian cancer cell line were determined as 25, 20, 15 µg/ml of dendrosomal curcumin and 250, 200, 150 µg/ml of oxaliplatin after 24, 48 and 72 hr, respectively by MTT test. And stability and mortality rate of encapsulated curcumin is extremely higher than normal curcumin. Also non-lethal activity of dendrosomal carrier was confirmed in IC50 dose of dendrosomal curcumin.

Conclusion: We showed that dendrosomal curcumin could be considered as a potential chemotherapeutic agent in ovarian cancer cancer such as oxaliplatin.

Key words: *Dendrosomal curcumin, Oxaliplatin, Ovarian cancer, MTT test.*

P-117

Analysis of pi3k signaling pathway indicates that cord blood hematopoietic stem cells (HSCS) aren't led to become cancerous after co-culture with mesenchymal stem cells (MSCS)

Amiri N[1], Shahhoseini M[2].
1. *Faculty of Basic Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: upepo@yahoo.com

Introduction: Umbilical cord blood transplantation (UCBT) is a medical procedure in the field of hematology. Umbilical cord blood (UCB) is a suitable HSC source for transplantation, but this process in adults is limited by the small number of HSC in each graft. Data derived from ex vivo co-culture systems using MSCs as a feeder cell layer suggest that cell-to-cell contact has a significant impact on the expansion of HSCs. This is likely that HSCs become cancerous when co-culture with MSCs.

Materials and Methods: In this study, the expression levels of three main genes of this signaling pathway were analyzed. we isolated HSCs from human umbilical cords purified by MACS method. Flow cytometery was performed with Anti-CD34 to validate the purified HSCs. Moreover, the adipose MSCs (ASCs) and bone marrow MSCs (BM MSCs) were verified by CD90, CD105 and CD73 markers.

Results: PI3K signaling pathway genes including PIK3CA, PIK3R1 and PTEN expression levels were analyzed by real-time PCR. The results indicated that the expression levels of PIK3CA, PIK3R1 and PTEN increased after co-culture with the two sources of MSCs.

Conclusion: It means, these genes were expressed in a harmonious statement, so HSCs after co-culture with MSCs, aren't led to become cancerous.

Key words: *Hematopoietic stem cells, Mesenchymal stem cells, Co culture, PI3K signaling pathway.*

P-118

The effect of mesenchymal stem cell (MSC) of bone marrow and fat tissue on expression of *p53* tumor suppressor gene in cord blood hematopoitic stem cell (HSC)

Zavari R[1, 2], Abroun S[2, 3], Favaedi R[4], Zarabi M[2, 5], Shahhoseini M[4].

1. *Faculty of Basic Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
2. *Royan Stem Cell Technology co. (Royan cord blood bank), Tehran, Iran.*
3. *Department of Hematology, Faculty of Medical Sciences, University of Tarbiat Modares, Tehran, Iran.*
4. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
5. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: zavari@yahoo.com

Introduction: Stem cell transplantation has been a wonderful stepping stone into the advancement of regenerative medicines. Umbilical cord blood (UCB), like bone marrow, is a rich source of hematopoietic stem (HSC) and progenitor (HPC) cells for treatment of a wide variety of malignant and non-malignant hematopoietic disorders. Since MSCs can act as a part of Nich and have effect on self-renewal, differentiation and life span of HSCs.
Materials and Methods: The cord blood samples were gifted from Royan Cord Blood Bank by familial permission. Then HSCs were purified by MACS method and confirmed by Flow cytometry, Flow cytometry analyze was performed with Anti-CD34. Furthermore, the adipose MSCs (ASCs) and bone marrow MSCs (BM MSCs) were characterized by CD90, CD105 and CD73 anti body. The presence of HSCs in UCBs mononuclear cells was evaluated via colony assay technique. Then, p53, p21 and MDM2 expression levels were analyzed by real-time PCR.
Results: The results indicated that the expression level of p53 increased in co-culture with MSCs which it means that HSCs were differentiated. Variations in p21 and MDM2 expressions, before and after co culture with MSCs, and the analysis of surface markers of HSCs via flow cytometry verified the results of the p53 expression level. Colony assay results showed that total number of CFCs increased after co culture with MSCs that it is consistent with flow cytometry results.
Conclusion: In conclusion the results show HSCs after co-culturing with MSCs tends to be differentiated than proliferate.
Key words: *Hematopoietic stem cells, Mesenchymal stem cell, Co culture, p53 genes, Expression.*

P-119

The effect of mesenchymal stem cell (MSC) of bone marrow and fat tissue on expression and epigenetic profile of *ID* genes family in cord blood hematopoitic stem cell (HSC): relying on CCAAT regulatory region

Mirzadeh Hosseini NS[1], Zavari R[2], Favaedi R[3], Zarabi M,[4] Abroun S[5], Shahhoseini M[3].

1. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Faculty of Basic Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
3. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
5. *Department of Hematology, Faculty of Medical Sciences, University of Tarbiat Modares, Tehran, Iran.*

Email: n_1368mirzadeh@yahoo.com

Introduction: Hematopoietic development is a complex process that can be control by expression of different genes including ID gene family. ID (inhibitors of differentiation) gene family has four members (ID1-ID4) and plays an important role in differentiation and specification of blood cells. These genes have regulatory CCAAT motif in their promoter. One of the proteins that interact with this motif is CEBPα transcription factor that control proliferation and cell differentiation. HSCs are multipotent progenitor cells that can produce all types of blood cells. To achieve these cells, umbilical cord blood has many advantages over other resources. The use of mesenchymal stem cells (MSCs) as a feeder layer, could provide an appropriate environment for growth and expansion of HSC of cord blood in vitro.
Materials and Methods: In this study, the cord blood samples were used with the consent of the individuals who registered in the Royan cord blood bank. HSCs were extracted from the umbilical cord blood and were purified by MACS method. Flow cytometry was performed with Anti-CD34 to validate the purified HSCs. Moreover, the adipose MSCs (ASCs) and bone marrow MSCs (BM MSCs) were verified by CD90, CD105 and CD73 markers. Then, ID1-ID4 expression levels were analyzed by real-time PCR. Finally, by using ChIP technique, the presence of the protein CEBPa in regulatory region of these genes was evaluated.
Results: Our study has shown that expression of ID1 and ID3 significantly decrease in HSC after co-culture with BM MSCs and ASCs, especially BM MSCs. About the presence of CEBPa protein, a significant increase of this protein factor in regulatory region of ID1 and ID3 in those HSCs that co-culture with ASCs were observed. Furthermore the results of flocytometry confirmed the results of epigenetic and gene expression.
Conclusion: Since previous studies showed that ID1 and ID3 act as negative regulators of HSCs differentiation, and by taking into account the results of epigenetic and gene expression, in conclusion it can be noted that fat tissue mesenchymal stem cells may be more suitable candidate for co-culture with HSCs of umbilical cord blood.
Key words: *Hematopoietic stem cells, Mesenchymal stem cell, Co-culture, ID genes, CEBPα transcription factor.*

P-120

Chondroblastic gene expression in mesenchymal stem cells derived from omentum tissue of NMRI mouse

Goudarzi Z.

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: negaar.goudarzi@gmail.com

Introduction: Mesenchyme is a type of undifferentiated loose connective tissue that is derived mostly from mesoderm. At the present time it has been proved that mesenchymal stem cells (MSCs) are able to divide into a variety of different cells as adult stem cells (ASCs). Mesenchymal stem cells derived from omentum tissue are pluripotent cells which have been identified recently.
Materials and Methods: For 21 days, omental mesenchymal stem cells of 10 NMRI mice were co-cultured with different concentrations of hyaline cartilage extracts for chondrogenic differentiation. At first to prove the mesenchyme being in cultured cells and then to show the differentiation of cells treated with cartilage extracts, expressions of mesenchymal-specific genes such as octamer-binding transcription factor-4 (Oct-4), Wilm's tumor suppressor gene-1 (WT-1) and cartilage-specific genes like aggrecan (AG), collagen type-II (Col2A1) and collagen type-X (Col10A1), were analyzed using reverse transcription polymerase chain reaction (RT-PCR).
Results: Mesenchymal stem cells derived from NMRI mouse omentum tissue expressed WT-1 gene as adult stem cell marker, Oct-4 as embryonic pluripotent stem cell marker and Actin Beta (ACTB) gene as housekeeping marker, of omentum tissue. OMSCs lost their spindle and fibroblastic morphology through differentiation, and changed to elliptical appearances. Messenger ribonucleic acid (mRNA) expression of cartilage-specific genes include AG, Col2A1and Col10A1 in differentiated cells were detected on day 21.
Conclusion: These observations showed that omental mesenchymal stem cells are capable to differentiate into chondrocytes in vitro by induction of hyalin cartilage tissue extract in absence of extracellular matrix.
Key words: *Mesenchymal stem cells, Omentum tissue, Chondrocyte, Expression of aggrecan, Col2A1, Col10A1.*

P-121
Screening of mutation in Ring-Finger and BRCT omains of *BRCA1* Ggene in Iranian patients with familial breast cancer

Miresmaeili SM[1], Kalantar SM[2].
1. *Department of Biology, Science and Art University, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductivev Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: smm230@gmail.com

Introduction: The most prevalent malignancy in women is Breast cancer (BC) and accounts for one third of all female cancers. In Iran, almost 50% of the breast cancer cases have diagnosed before the age of 50 years. The breast and ovarian cancer susceptibility gene 1 (*BRCA1*) mutations accounts for about 40-45% of hereditary breast cancer cases. Familial aggregation is thought to account for 5-10% of all breast cancer cases, and germline mutations in *BRCA1* and *BRCA2* account for less of the half of these inherited cases. The RING domain of BRCA1 has an E3 ubiquitin ligase function. The BRCT domains are involved in the processes of DNA damage checkpoint and DNA repair. Mutation in both domains results in increased risk of breast cancer.
Materials and Methods: In order to study *BRCA1* mutation spectra in the Iranian population, 107 patients with a reported family history of breast and/or ovarian cancers were tested. Screening for mutation in the RING and BRCT domains in *BRCA1* gene was carried out by the polymerase chain reaction-based on single-stranded conformational polymorphism (PCR-SSCP) and direct DNA sequencing.
Results: All of patient underwent surgery and had familial history of breast and/or ovarian cancer. All the cases were diagnosed under the age of 54 yr. Patients' mean age at the time of diagnosis was 46.14 yr (range: 33-51). After sequencing, we found 13 novel missense mutations in patients. The most mutations were found in Ring- finger domain. The novel mutations were considered to be of unknown clinical significance according to BIC databases. In silico studies predict that several variants affects on protein stability and possibly damaging.
Conclusion: In conclusion, inherited mutations in the *BRCA1* gene are constituted for the major hereditary breast cancer cases. Deficiency in BRCA1 protein is considered in high risk family with breast cancer. Screening and detection of mutations in *BRCA1* gene may help in medical management of patients and their families.
Key words: *Ovary cancer, Breast cancer, BRCA1, Mutation, PCR-SSCP, Direct Sequencing.*

P-122
Distribution of estrogen receptor alfa gene -397 T>C polymorphisms among women with polycystic ovary syndrome

Golzade M[1, 2], Montazeri F[2], Kalantar SM[3], Sheikhha MH[3], Dehghani MR[2], Karimi R[1, 2].
1. *Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: Golzade_60@yahoo.com

Introduction: Polycystic ovarian syndrome(PCOS) is the most common endocrine and a complex heterogeneous disorder of women in their reproductive age. Recently the number of genes involved in susceptibility to PCOS increased dramatically, each

with individually effect which interacts with one another.

Materials and Methods: Polycystic ovary syndrome patients (n=50) and controls (n=50) that referred to Yazd Research and Clinical Center for Infertility were studied. DNA extraction and ARMS- PCR were used to detect the polymorphic genotypes. Chi-square test and the frequency differences of alleles and genotypes between two groups were compared.

Results: The primary results suggest that CC genotype of the *ESR1* rs2234693 polymorphism is significantly associated with an increased risk of PCOS. (non-TT genotype were 22% for patients with PCOS and 46.6% for controls). For confirming this association, study continues on a larger number of PCOS and control samples.

Conclusion: The *ESR1* gene -397 T>C polymorphism may be associated with pathophysiologic aberrancies involved in PCOS.

Key words: *Estrogen receptor alfa gene, Polymorphisms, ARMS- PCR, PCOs*

P-123

Association between interleukin 4 gene seventy-base-pair variable number of tandem repeats polymorphism and uterine leiomyoma

Mohammadoo-Khorasani M, Moossavi M

1. *Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran.*
2. *Genomic Research Center, Birjand University of Medical Sciences, Birjand, Iran.*

Email: miladkh24@yahoo.com

Introduction: Uterine Leiomyoma (UL) is the most common gynecological tumor and a public health problem. Higher serum interleukin 4 (IL4) level, as an anti-inflammatory cytokine that regulates TH1/TH2 cells balance, has been observed in the uterine cavity. Objectives: The aim of this study was to investigate the association between *IL4* gene variable number of tandem repeats (VNTR) polymorphism and the risk of UL in southeast of Iran.

Materials and Methods: We compared 99 patients with UL with 102 healthy controls. The *IL4* VNTR polymorphism was genotyped by gel electrophoresis after PCR amplification.

Results: There was no significant association between RP*1/RP*2 and RP*2/RP*2 genotypes and UL; however, a significant association between RP*2/RP*2 genotype and UL was found after adjustment for age (OR, 4; 95% CI, 1.3-12.4; p=0.015). The frequency of RP*2 allele was significantly higher in women with UL (OR, 1.9; 95% CI, 1.1-3.5; p=0.03).

Conclusion: The *IL4* VNTR RP*2/RP*2 genotype could be an age-related risk factor for UL. Moreover, the frequency of RP*2 allele was significantly higher in women with UL.

Key words: *Interleukin-4, Minisatellite Repeats, Polymorphism, Genetic.*

P-124

Detection of novel mutations in exon 20 of the *BRCA1* gene in a patient with familial breast and ovarian cancer

Miresmaeili SM[1], Kalantar SM[2].

1. *Department of Biology, Science and Art University, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: smm230@gmail.com

Introduction: Hereditary ovarian and breast cancer due to mutations in the *BRCA1* and *BRCA2* genes is the most common cause of hereditary forms of both ovarian and breast cancer. The lifetime risk of having ovarian cancer is about 40% in *BRCA1* mutation carriers. *BRCA1* is a tumour suppressor gene that is involved in DNA-damage repair. About 20-25% of all breast cancer cases have the family history. *BRCA1* gene consists of 24 exons that encode a protein with 1863 amino acids. Exon 11 is the largest exons and most of the disease-linked mutations have been found in it.

Materials and Methods: A total of 107 breast and/or ovarian cancer patients and 93 unrelated healthy women with no clinical phenotype of any malignancy or familial cancer history constitute the study groups. After primer designing for functional exons, evaluation of mutations performed by PCR-SSCP technique followed by direct sequencing. mutation effects on protein structure and function were predicted by PolyPhen-2 and I-Mutant Suite software.

Results: In this study, two novel missense mutations c.3059C>T (p.Pro1020Leu) and c.3074C>T (p.Thr1025Ile) have been detected in *BRCA1* exon11 by PCR-SSCP technique followed by direct sequencing. In silico Analysis of c.3074C>T mutation on protein function predict that this substitution affects on protein structure and is predicted to be possibly damaging.

Conclusion: Mutation screening in Medical management of *BRCA1* gene may help in medical management of patients and their families.

Key words: *Ovarian cancer, Breast cancer, BRCA1, DNA sequencing, Exon11, Mutation analysis, Familial history.*

P-125

Expression of placental growth factor mRNA in preeclampsia

Nikuei P[1], Rajaei M[2], Malekzadeh K[1, 3], Nejatizadeh A[1, 3], , Mohseni F[1], Poordarvishi F[1], Ghashghaeezadeh N[2], Mohtarami M[4].

1. *Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
2. *Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
3. *Department of Medical Genetics; Faculty of Medicine; Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*

4. *Biology Department, Long Island University, Post Campus, New York, USA.*
Email: rajaei_minoo@yahoo.com; kianoosh.malekzadeh@hums.ac.ir

Introduction: Preeclampsia (PE) is a serious complication of pregnancy with hallmarks of incomplete placentation, placental ischemia and endothelial dysfunction. Imbalance between vascular endothelial growth factor (VEGF), placenta growth factor (PlGF) and their receptors play important role in pathophysiology of PE. This study was aimed to asses PlGF mRNA expression in placenta of women affected with PE.
Materials and Methods: In this cross-sectional study, expression of PlGF mRNA was evaluated in 26 mild PE cases, 15 severe preeclamptic women and 20 normotensive controls. Patients were sub-classified as early onset PE (9) and late onset (32). After RNA extraction, PlGF expression was quantified with qRT-PCR.
Results: The results of PlGF mRNA expression between mild-severe, and early-late onset PE patients showed no statistically significant difference compared with the control group (p=0.661, p=0.205 respectively).
Conclusion: Despite we found no distinct differential expression of PlGF mRNA in placental tissue of PE patients compared with control women, but according to decreased level of this angiogenic factor in PE even before clinical onset of the disease, determining molecular mechanisms related to reduced secretion of PlGF into the maternal circulation may be useful for future therapeutics.
***Key words**: Preeclampsia, Expression, PlGF, Endothelial dysfunction*

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The serum level of transforming growth factor beta1 and its association with *Foxp3* gene polymorphism in Iranian women with recurrent spontaneous abortion

Hadinedoushan H[1], Abbasirad N[1], Aflatoonian A[2], Eslami G[3].
1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Microbiology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: hhadin@ssu.ac.ir

Introduction: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive miscarriages, which affects up to 3% of couples trying to establish a family. It has been postulated that a proportion of these repeated pregnancy losses might be due to immune causes.
Materials and Methods: Eighty women with RSA were compared with eighty in a control group. Serum levels of TGF-β1 were measured using ELISA and Foxp3 (rs3761548) promoter polymorphisms using a PCR-RFLP technique. In addition, serum levels of TGF-β1 were compared in different genotypes in the two groups.
Results: The women's ages in the two groups were similar (30.15±4.42 yr [RSA] vs. 29.97±4.51 [control]) as were serum TGF-β1 concentrations in case and control groups (53.42±2.08 ng/ml in control and 56.31±2.58 ng/ml in the RSA group; p=0.4). Furthermore, there was no significant difference in the genotype frequencies of the rs3761548 *Foxp3* gene between the two groups (p=0.3) and the levels of TGF-β1 were similar in different genotypes.
Conclusion: The data indicate that serum *TGF-β1* levels and *Foxp3* (rs3761548) promoter polymorphism is not a risk factor for RSA and that there is no association between the polymorphism and serum TGF-β1 levels.
***Key words:** Foxp3 gene, T regulatory, TGF-β1, Polymorphism, Recurrent abortion.*

P-127
Evaluation of soluble human leukocyte antigen-G in pripheral blood of pregnant women with gestational diabetes melitus

Sadatshobeiri S, Abediankenari S.
Department of Immunology, Mazandaran University of Medical Sciences, Sari, Iran.
Email: saeideshobeiri@gmail.com

Introduction: Research says that diabetes may develop in over 10% of non-diabetic pregnant women. Diabetes which generally occurs late in second trimester and third trimester of pregnancy, it is called gestational diabetes. Overweight or suffering from obesity before pregnancy is type 2 diabetes risk factor. In most cases, diabetic symptoms disappear after delivery. HLA-G has an important role both in mother and fetus tolerance during pregnancy, it may also be effective in the protection of pancreatic islet cells.
Materials and Methods: In this case-control study, we measured serum HLA-G levels in 24 pregnant women with gestational diabetes compared with 30 normal pregnant women using sandwich ELISA.
Results: HLA-G levels were significantly low in pregnant women with gestational diabetes in contrast to normal pregnant women (p=0.001).
Conclusion: In this study, we found that HLA-G levels were reduced in women with gestational diabetes compared with control group. Therefore, it is suggested that measurement of HLA-G in pregnant women can be considered as an indicator in prognosis of gestational diabetes.
***Key words:** HLA-G, Gestational diabetes, Pregnancy.*

P-128
Comparison of the immunomodulatory properties of root and leaves of Arctium lappa (Burdock) in vitro

Behnamfar M.
Student Research Committee, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.
Email: morteza.behnamfar@yahoo.com

Introduction: The roots and leaves of Arctium lappa (Burdock) have been used for different therapeutic purposes, especially for diseases linked to chronic inflammation.
Materials and Methods: PHA- or LPS-stimulated splenocytes were treated with different concentrations of root or leaves extract of burdock and proliferation of splenocytes measured by MTT assay. The levels of IFN-γ and IL4 in the supernatants of PHA-stimulated splenocytes determined using ELISA. We also studied the effects of root and leaves extract of Burdock on Nitric Oxide production by LPS-stimulated macrophages using the Griess reagent.
Results: Our findings showed that both root and leaves extract of burdock have suppressive effects on LPS-stimulated splenocytes proliferation, IL-4 secretion from PHA-stimulated splenocytes, and NO production from LPS-stimulated macrophage and stimulatory effects on PHA-stimulated splenocytes proliferation, and IFN-γ secretion from PHA-stimulated splenocytes. Although both root and leaves extract of burdock had similar immunomodulatory effects in vitro, stronger immunomodulatory effects seen in root extract of burdock.
Conclusion: According to our results, we suggest that root of burdock is better option than leaves of burdock in modulation immune responses and inflammations.
Key words: *Arctium lappa, Burdock, Immunomodulation, Macrophage, Nitric Oxide.*

P-129
Immunopathogenesis of preterm labor disease

Khzaei HA[1], Tymori B[2], Mohmmadi M[3], Khazaei A[4], Khazaei B[4], Roudbari HA[1], Hejazenia F[1], Toufigh N[4].
1. *Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.*
2. *Department of Gynecology, Zahedan University of Medical Sciences, Zahedan, Iran.*
3. *Department of Biostatic, Zahedan University of Medical Sciences, Zahedan, Iran.*
4. *Zahedan University of Medical Sciences, Zahedan, Iran.*

Email: k_khazaei118@yahoo.com

Introduction: Preterm birth refers as the end of the pregnancy before thirty-seventh weeks, which causes problems for the newborn. Based on report of WHO, low birth weight of infant and intrauterine growth retardation factors that are the predictors factors of infant mortality in the first 28 days of life index have been proposed to the preterm birth. As several immunological and non-immunological factors are effective in this disease, therefore we designed this study with the aim of any relationship with preterm delivery and possible role of the patient's some immunological factors.
Materials and Methods: 120 patients with preterm labor and healthy asymptomatic preterm delivery women were selected for study by cross sectional case-control method. After performing diagnostic tests, laboratory variables including differences in frequency of serum levels of CRP, IgG, IgM and C3 complement of the two groups were compared and the results were analyzed using X^2 test by SPSS software.
Results: The age of patients ranged were between 19-35 yr with a mean of 6.4±8.25 yr. Statistically significant differences in serum levels of CRP, IgG, IgM and C3 complement was observed between the two groups (p=0.02) while significant differences in serum levels of IgA, IgE and C4 complement was not observed (p=0.82).
Conclusion: Our results showed that the process of immunological factors have been effective factors in preterm labor disease that controlling these factors are predictive in prognosis of disease.
Key words: *Preterm labor disease, IgG, IgE, IgM, IgA, C3, CRP, C4.*

P-130
Serological study of Toxoplasmosis in hemodialosis patients using ELISA, PCR and Chemiluminescence techniques

Feiz Haddad MH[1], Kazemi F[2].
1. *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
2. *Department of Parasitology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

Email: hhadi_2000@hotmail.com

Introduction: Determining the prevalence of anti-Toxoplasma antibodies before becoming pregnant is an effective measure to detect positive rate of Toxoplasma IgM and IgG antibodies. Due to toxoplasmosis complications, including miscarriage, premature birth, pathological changes in central nervous system, prevention of congenital infection is essential. Given the high prevalence of toxoplasmosis in the world and high abortion with significant anomalies thus, this parasite should be investigated in a particular way. Notice of the female population of childbearing age is necessary especially if they are unsafe against Toxoplasma parasite. On the other hand, this parasite can produce irreparable effects in patients with impaired renal function and immunosuppressive individuals. Since, Toxoplama in these patients are high and they might be in high risk of severe damage, therefore, this study could help authorities in planning of control and prevention of parasite.
Materials and Methods: DNA assay along with anti-toxoplasma IgM and IgG, applying ELISA and

Chemiluminescence methods to detect Toxoplasma gondii in kidney failure patients. Amount of five ml blood was taken, centrifuged and 2 ml of isolated serum placed in micro-tubes and kept in -20°C until testing. The pellet leucocyte-rich supernatant was kept in 2 ml tubes at -80°C for PCR experiment. The diagnostic ABON (UK) kit was used to measure Toxoplasma IgG and IgM evaluating through Ab Capture ELISA and Indirect ELISA methods. For statistical analysis, Chi-square tests and SPPS software version 21 were used.

Results: ELISA experiments showed a total of 57 positive IgG out of 160 subjects in two groups of hemodialysis patients and control group however, this was positive in 38 subjects applying chemiluminescence method. In fact, false positive subjects were 19 cases in ELISA, which was null using Chemiluminescence method. Detected IgM antibodies were 9 cases by ELISA while this was 8 in Chemiluminescence method. PCR indicated just two positive for parasite in 80 blood samples of patients however, it was null in control group.

Conclusion: The prevalence of Toxoplasma antibodies in patients was higher than control group. Chemiluminescence method compared to ELISA was more accurate and sensitive with very low false positives. In fact, IgM and IgG in patients and healthy individuals for a total review of 20 cases were positive in ELISA, where there was one negative in Chemiluminescence method. Immunological methods often not applicable to differentiate latest infections from older subjects and T. gondii-specific IgM could remain detectable for more than 1 year after primary infection therefore, PCR was applied to detect T. gondii parasite in Duffy part of blood samples to detect recent T. gondii infection.

***Key words**: ELISA, PCR, Chemiluminescence, Toxoplasmosis, Hemodialysis.*

P-131

Survey of *CTLA-4* gene polymorphism in women with recurrent pregnancy loss

Vaziri Nezamdoust F[1], Hadinedoushan H[1], Ghasemi N[2].

1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fereshteh.vaziri@gmail.com

Introduction: Recurrent pregnancy loss (RPL) is one of the most important pregnancy problems. Epidemiologic studies have revealed that 1-2% of women experience recurrent pregnancy loss. An aberrant regulation of immunological, metabolic, vascular and endocrine processes leads to RPL. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expresses on active T cells and causes of inhabitation and toleration of active T cells. *CTLA-4* gene expression in fibroblast cells of the placenta throughout pregnancy and during pregnancy is increased and causes to tolerance during pregnancy.

Materials and Methods: This study was carried out on two groups of women who were referred to the Yazd Reproductive Sciences Institute.150 women with a history of at least 2 RPL as the case group and 150 women who didn't have a history of abortion with at least one healthy child as the control group. Five milliliter peripheral blood was obtained from all samples and then DNA was extracted. A SNP rs 3087243 of *CTLA-4* gene was analyzed using the RFLP-PCR method.

Results: There was no difference between the two groups regarding age of women (26.7±3.5 vs. 28.6±3.6 yr). In the case group, the genotype frequencies of rs 3087243 polymorphisms were AG (46%), AA (24%), and GG (30%), and in the control group, they were AG (59.7%), AA (23.3%), and GG (17.3%). There was a significant difference between the genotypes of AA, AG, and GG in two groups (p=0.022).

Conclusion: Our result indicates that the rs 3087243 AG genotype may contribute to RPL development in Iranian women.

***Key words:** Recurrent pregnancy loss, Single nucleotide polymorphisms, CTLA-4 gene.*

P-132

Successful treatment of recurrent spontaneous abortions (RSA) patients with elevated natural killer cells with intravenous immunoglobulin (IVIG)

Bahrami asl Z[1, 2], Ahmadi M[3, 4, 5], Farzadi L[1, 2], Ghasemzadeh A[1, 2], Hamdi K[1, 2], Babaloo Z[3, 4], Nouri M[1, 2], Yousefi M[3, 4].

1. *Department of Reproductive Biology, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Woman's Reproductive Health Research Center, Tabriz, Iran.*
3. *Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran.*
4. *Drug Applied Research Center, Tabriz University of Medical Science, Tabriz, Iran.*
5. *Student's Research Committee, Tabriz University of Medical Science, Tabriz, Iran.*

Email: dr.za.bahrami@gmail.com

Introduction: Recurrent spontaneous abortions (RSA) has a multifactorial etiology, mainly due to karyotype abnormalities including balanced translocation, anatomical uterine disorders, and immunological factors, although in 50-60% the etiology is unexplained. The treatment of RSA remains challenging, and the role of intravenous immunoglobulin (IVIG) in RSA is controversial.

Materials and Methods: 44 women with a history of three or more recurrent abortion were included and peripheral blood was drawn upon positive pregnancy test. On the same date, IVIG, 400 mg/kg, was administrated intravenously and continued every 4 wk through 28-30 wk of gestation. 12 RSA patients were

included as IVIG untreated group. We investigated IVIG effect on NK cell percentage in RSA patients before and after treatment.

Results: NK cell percentage was reduced from 18±0.23% to 7±0.18% in IVIG treated patient (p=0.001) after 28-32 wk. Pregnancy outcome after IVIG treated was significantly higher than untreated patient (p=0.004).

Conclusion: High levels of NK cells are detected in women affected by RSA. IVIGs are capable of decreasing them with a long-term efficacy. Also our findings shed more light on the mechanisms of IVIG immunomdulatory effects and introduce IVIG as a promising therapeutic approach in RSA patients.

***Key words**: Recurrent spontaneous abortion, Intravenous immunoglobulin G, Treatment, NK cell.*

P-133
Characterization of ascorbic acid, dexamethasone and tissue extract on omental mesenchymal stem cells differentiation

Goudarzi Z.
Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: negaar.goudarzi@gmail.com

Introduction: Mesenchymal stem cells (MSCs) retain the capacity to proliferate and to differentiate along multiple connective tissue lineages. Omental mesenchymal stem cells (OMSCs) are considered as a favorable cell choice due to their multipotent differentiation capability. However, cellular responses to various chemical and biomaterial-elicited stimulates are still poorly understood.

Materials and Methods: 12 days old NMRI mice have been used as samples. After the isolation and confirmation of OMSCs, they were co-cultured with cartilage extracts and the chondrogenic differentiation was monitored. Expressions of mesenchymal stem cell markers were analyzed via flow cytometry. Moreover, DEX and ascorbic acid were added to the medium and changes were analyzed. Furthermore, immunocytochemistry was performed for cartilage matrix protein collagen type-II (CT-II) production. Cartilage matrix-sulfated proteoglycans (PGs) production was determined via toluidine blue and alcian blue staining.

Results: The phenotypic characterization revealed the positive expression of CD90, CD44 and the negative expression of CD45 in OMSCs. Chondrogenic differentiation at presence of 30µg/ml cartilage extract was proved by immunohistochemistry on day 21. DEX treatment in the presence of cartilage extract up-regulated the expression of CT-II genes and PGs which were assessed by immunohistochemistry, alcian blue and toluidine blue staining. The presence of dexamethasone and ascorbic in the absence of cartilage extract led OMSCs to differentiate into neuron-like cells. OMSCs lost their spindle shape, while growing long excrescences and nodes.

Conclusion: These observations suggest that cartilage extract without any association is able to induce differentiation of OMSCs into chondrocytes. Additionally, DEX may play an important role in the maintenance of cartilage homeostasis.

***Key words:** Mesenchymal stem cells, Omentum tissue, Differentiation, Cartilage extract, Dexamethasone, Ascorbic acid, Neural cells, Chondrocyte.*

P-134
Insulin resistance (*RETN*) gene polymorphism and polycystic ovary syndrome

Hamrahie-Michak M[1], Ali-Hassanzadeh M[2], Gharesi-Fard B[3], Sadri M[4].
1. *Department of Biochemistry, Payame Noor University, Taft, Yazd, Iran.*
2. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
4. *Department of Immunology, Iran University of Medical Sciences, Tehran, Iran.*

Email: smim8035@yahoo.com

Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorder of women at pregnancy age. The major features of PCOS include anovulation, Infertility, Obesity and metabolic syndrome, Gestational Diabetes, type 2 diabetes and hyperandrogenism.

Materials and Methods: 100 patients with PCOS and 98 healthy women were recruited from Motahari Clinic, a sub-branch of Shiraz University of Medical Sciences. Patients were all between 18 and 35 years of age. The *RETN* gene polymorphism in -420 C/G and +299 A/G positions were genotyped by PCR-RFLP technique.

Results: No differences were observed in -420 C/G and +299 A/G positions in PCOS compared to the control groups.

Conclusion: Our obtained results suggest that there is no association between these two single nucleotide polymorphisms and pathogenesis of POCS.

***Key words:** Polycystic ovary syndrome, Insulin resistance (RETN), Polymorphism.*

P-135
Intravenous immunoglobulin (IVIG) modulates natural killer cell and improves live birth rate in women with recurrent pregnancy loss

Aghebati-Maleki L[1], Ahmadi M[1], Nouri M[2], Babaloo Z[1], Hamdi K[2], Yousefi M[1].
1. *Immunology, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Reproductive Biology, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

Email: leili_aghebati_maleki@yahoo.com

Introduction: Recurrent pregnancy loss (RPL) has a multifactorial etiology, mainly due to karyotype abnormalities including balanced translocation, anatomical uterine disorders, and immunological factors, although in 50-0% the etiology is unexplained. The treatment of RPL remains challenging, and the role of intravenous immunoglobulin (IVIG) in RPL is controversial.
Materials and Methods: 32 women with a history of three or more recurrent abortion were included and peripheral bloods were drawn upon positive pregnancy test. On the same date, IVIG, 400 mg/kg, was administrated intravenously and continue every 4 weeks through 28-30 wk of gestation. 12 RPL patients were included as IVIG untreated group.
Results: We investigated IVIG effect on NK cell percentage and activity in RSA patients before and after treatment. NK cell percentage was reduced from 18±0.23 to 7±0.18 in IVIG treated patient ($p \leq 0.0001$) after 28-32 weeks. NK cell cytotoxicity was also decreased from 19.25±3.860 to 13.22±2.802 ($p \leq 0.0001$) in IVIG treated group. Pregnancy outcome after IVIG treated was significantly higher than untreated patient ($p=0.004$).
Conclusion: Our findings suggest that the mode of action of IVIG in the prevention of recurrent pregnancy loss (RPL) may be through a modulate in natural killer (NK) cell percentage and cytotoxicity.
Key words: *Recurrent Pregnancy Loss, Intravenous immunoglobulin G, Treatment, NK cell, NK cell cytotoxicity.*

P-136

The association between single nucleotide polymorphism in interleukin-27 gene and recurrent pregnancy loss in Iranian women.

Nematollahi Z[1], Hadinedoushan H[1], Aflatoonian A[2], Eslami G[3], Ghasemi N[4].
1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Yazd Reproductive Sciences Institute, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Micology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Department of Genetic, Reccurent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: z.nematollagi@yahoo.com

Introduction: Recurrent pregnancy loss (RPL) has been defined as two or more miscarriages before 20th week of gestation. It seems that IL-27 may reduce inflammatory responses and affect the survival of the embryo during human pregnancy. IL-27 polymorphisms may influence RPL by altering the levels or the activity of gene product.
Materials and Methods: A case-controlled study was performed on two groups consisting of 150 healthy women with at least one delivery (control group) and 150 women with two or more primary RPLs history (RPL group). The -964 A>G SNP in *IL-27* gene was determined by PCR-RFLP technique. Genotype and allele frequencies were compared using T test between two groups.
Results: There was no difference between the two groups regarding age of women (29±4.4 [control] vs. 30.84±5.2 yr [case]). In the RPL group, the genotype frequencies of -964 A>G polymorphism were AG (49.3%), AA (40%), and GG (10.7%), and in the control group, they were AG (43.3%), AA (48.7%), and GG (8%). There was no significant difference between the genotypes of AA, AG, and GG in two groups ($p=0.23$). As the frequency of allele A was 64.7% in the RPL group and 70.3% in the control group, the difference in frequency of allele A in -964 A>G between two groups was not significant ($p=0.19$).
Conclusion: Our findings indicate that SNP of -964 A>G in *IL-27* gene is not a risk factor for RPL in Iranian women.
Key words: *Cytokine, IL-27, Inflammation, Polymorphism, Recurrent abortion.*

P-137

Association between *HLA-E* gene polymorphism and unexplained recurrent spontaneous abortion (RSA) in Iranian women

Fotoohi M.
Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: maryam.fotoohi91@yahoo.com

Introduction: Human leukocyte antigen-E (HLA-E) is a non-classical major histocompatibility complex (MHC) class I antigens which expressed on extra villous cytotrophoblast, which interacts with NKG2A, is an inhibitory receptor on natural killer (NK) cells and leading to down regulation of immune response in the maternal-fetal interface and provides maternal immune tolerance of the fetus.
Materials and Methods: Amplification Refractory Mutation System (ARMS-PCR) technique was carried out to detect polymorphism in exon 3 of the *HLA-E* gene in women with RSA and controls (n=200). Differences between groups were analyzed by SPSS19 software using χ^2 test.
Results: There was no significant difference in the allele frequencies of the *HLA-E* polymorphism between RSA and fertile controls but HLA-E 0101/ 0103 heterozygous genotype was found to be significantly higher in RSA group ($p=0.006$, OR=1.73), so this genotype might confer susceptibility to RSA.
Conclusion: Our results suggest that HLA-E 0101/ 0103 heterozygous genotype leads to increase of RSA risk. It seems that by genotyping of *HLA-E* polymorphism, we can predict the risk of RSA in infertile women.
Key words: *Spontaneous abortions, HLA-E antigen, Polymorphism.*

P-138

Evaluation of the immunomodulatory effects of ethanolic extract of Trehala manna produced on Echinops persicus

Namdar Ahmadabad H[1], Behnamfar M[2].
1. *Department of Pathobiology and Medical Laboratory Science, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.*
2. *Student Research Committee, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.*

Email: morteza.behnamfar@yahoo.com

Introduction: In this study, the effects of Trehala manna extract evaluated on murine splenocyte and peritoneal macrophage in vitro.
Materials and Methods: For this purpose, PHA- or LPS stimulated-splenocytes were treated with Trehala manna extract and cell proliferation and also cytokine production from PHA-stimulated splenocytes determined by MTT assay and ELISA, respectively. We also evaluated the effect of this extract on activity and nitric oxide (NO) production from LPS-stimulated macrophages by MTT assay and Griess reaction, respectively.
Results: Our results showed that Trehala manna extract significantly increase PHA- and LPS-stimulated splenocytes proliferation and IFN-γ production but no IL-4 from PHA-stimulated splenocytes. We also found that this extract significantly increase activity and NO production from LPS-stimulated macrophages.
Conclusion: Taken together, these results suggest that Trehala manna extract have immunostimulatory effect on splenocytes and macrophages.
Key words: *Immunomodulatory, Macrophage, Nitric Oxide, Shekar tighal, Trehala manna.*

P-139
Association of the rs2476601 single-nucleotide polymorphism with recurrent pregnancy loss (RPL)

Khanbarari F[1], Samadi M[2], Ghasemi N[2], Zare F[2].
1. *Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fatemeh.khanbarari@gmail.com

Introduction: One of the fundamentally important mechanisms for signal transduction is the Tyrosine Phosphorylation which plays a vital role in the regulation of the cells and govern a wide variety of biological processes. Among 110 protein-tyrosine-phosphatase (PTP) that have been identified in the human phosphoproteome, at least half of them are expressed in lymphocytes. Lymphoid-Tyrosine-Phosphatase (LYP) which is encoded by *PTPN22* gene, is one of the PTP enzymes which is expressed exclusively in immune cells and plays very important role in homeostasis of immune responses by inhibiting the signaling of immune-cell receptors. It has been clearly identified that the rs2476601 polymorphism in the *PTPN22* gene at position 1858 determines a R620W substitution and results in a gain-of-function form of the LYP enzyme which consequently leads to the stronger suppression of the early T cell activation processes. as the balanced immune response is important during pregnancy we analysed the frequency of rs2476601 in recurrent pregnancy loss patients and control group.
Materials and Methods: We had selected 200 patients with RPL as well as 200 normal individuals (control group) at the infertility center in Yazd and screened the rs2476601 variant with *PTPN22* PCR-RFLF method. Genotypes frequencies in RPL women and the fertile control group were compared using a Chi-square test.
Results: The association between RPL and the *PTPN22* T allele was confirmed by the p=0.018.
Conclusion: It revealed the effect of *PTPN22* variation in the genetic predisposition to RPL.
Key words: *Protein-tyrosine-phosphatase, Lymphoid-Tyrosine-Phosphatase (LYP), Recurrent Pregnancy Loss (RPL), PTPN22.*

P-140
A novel polymorphism of *PTPN22* And recurrent pregnancy loss (RPL)

Khanbarari F[1], Samadi M[1], Ghasemi N[2].
1. *Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Recurrent Abortion Research Center, Yazd treproductive Sciences Indtitute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: fatemeh.khanbarari@gmail.com

Introduction: Recurrent pregnancy loss (RPL) is a common complication of gestation which occurs in almost 1% of pregnancies and determined as the loss of three or more consecutive pregnancies, several factors are involved in causing these disorders such as uterine anomalies, endocrine dysfunction, chromosomal abnormalities and infection. As the fetus carries half of genetic material from the father so the maternal immune system must adapt to the presence of foreign Ag to continue pregnancy and prevent the fetus rejection. Any disorder in immune system function can lead to fetus rejection. Lymphoid-Tyrosine-Phosphatase (LYP) which is encoded by *PTPN22* gene plays a pivotal role in regulation of immune responses. Recently, the polymorphism of -1123G/C, located in the promoter region of the *PTPN22* gene was discovered that may change the gene expression. This SNP is associated with autoimmune disease such as Rheumatoid Arthritis and Diabetes as the balanced immune response is important during pregnancy we analysed the frequency of -1123C polymorphism in recurrent pregnancy loss patients and control group.
Materials and Methods: Polymerase chain Reaction-restriction fragment length polymorphism (PCR-RFLF) method was carried out to detect -1123C variant of the *PTPN22* gene in women with RPL and controls (n=200).
Results: The frequency of -1123C allele was higher in RPL patients as compared with fertile controls, but it wasn't significant.
Conclusion: Analysis of -1123C polymorphism suggested no association with RPL.
Key words: *Recurrent Pregnancy Loss (RPL), Lymphoid-Tyrosine-Phosphatase (LYP), PTPN22.*

P-141
OX40 and OX40L mRNA expression levels in blood cells of women with recurrent miscarriage

Rahmani F[1], Hadinedoushan H[1], Ghasemi N[2], Zare F[1].
1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences,Yazd, Iran.*

Email: fateme_rahmani_2014@yahoo.com

Introduction: Recurrent miscarriage (RM), a common complication of pregnancy, accounts for approximately 15% of clinically recognized pregnancies. Fifty percent of RM occur frequently for obvious reasons, but more than half of them without reason and probably occurs with immunological reasons.The costimulatory molecules OX40 and OX40L transfer a potent co-stimulatory signal to effectors T cells. OX40 is likely predominantly expressed on activated T cells, but this includes CD4 and CD8 T cells, Th2, Th1, and Th17 cells, as well as Foxp3+CD4+ regulatory T cells (Tregs).
Materials and Methods: This case-control study was carried out on two groups of women who were referred to the Yazd Reproductive Sciences Institute. Forty women with a history of RM who had at least two RM as case group and forty women with no history of abortion as the control group. 5ml of peripheral blood was obtained from all subjects, and immediately RNA was extracted and then cDNAwas synthesized. Evaluation of gene expression of OX40 and OX40L in twenty samples in each group was performed using Real-time PCR method.
Results: The mean age of subjects was the same in two groups (30.1±4.28 years). The mean abortion in case group was 4±1.5 (range 2-6). The expression levels of OX40 and OX40L genes do not show any significant difference between the two groups (p>0.05).
Conclusion: Our data indicate that expression levels of *OX40* and *OX40L* genes were similar between women with a history of RM and the control groups. After evaluation of all subjects, final results will be reported.
Key words: *Recurrent Miscarriage, OX40 gene, OX40L gene, Real-time PCR.*

P-142
The balance of the immune system between HLA-G and NK cells in unexplained recurrent spontaneous abortion and polymorphisms analysis

Arjmand F.
Department of Molecular Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
Email: fateme.arjmand@yahoo.com

Introduction: Human leukocyte antigen (HLA)-G is involvedin immunoregulatory processes and particularly in pathogenesis of inflammatory disorders such as recurrent spontaneouabortions (RSA).
Materials and Methods: enomic DNA from 117 RSA patients and 11 normal fertile control individuals was isolated using the salted out method. The two single nucleotide polymorphisms in *HLA-G* gene were analyzed using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). Differences between the two groups were analyzed by SPSS19 software using Chi-square test.
Results: The results revealed a significant increase in HLA-G*0105N allele in the proportion of whole group of RSA women compared with fertile controls (p=0.015), OR (95% CI)= 2.054 (1.798-2.347), as well as an absence of homozygosity for HLA-G*0105N in the study population. No significant difference was observed between the RSA and the fertile groups in terms of alleles and genotypes frequency of rs1736936 (p=0.323), OR (95% CI)= 1.056 (0.844-1.319).
Conclusion: The presented data suggest that the investigated HLA-G*0105N allele is potentially associated with RSA through linkage disequilibrium with other genetic elements. Meanwhile, the rs1736936 SNP do not predispose to RSA in the study population.
Key words: *Promoter polymorphism, rs1736936, HLA-G, Recurrent miscarriage, Recurrent spontaneous abortion, HLA-G*0105N.*

P-143
The immunomodulatory effects of probiotics on recurrent spontaneous abortion

Vahidi Z, Abdollahi E, Samadi M.
Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: ze.vahidi@gmail.com

Recurrent spontaneous abortion (RSA) is defined as a sequence of three or more consecutive pregnancies ending as a miscarriage before 20 wks of gestation. It happens in approximately 1-5% of women in reproductive age. RSA depends on numerous factors such as genetic, anatomic, endocrinologic, immunologic and microbiologic aspects. Probiotics are nonpathogenic microorganisms that well known for their protective effects in human immune mediated diseases via enhancing immune homeostasis through altering bacterial balance and interaction with the immune system or modulation of immune responses. The therapeutic effects of probiotics in the prevention and treatment of diseases have been found to be primary attributed to enhance the inflammatory responses and increasing non-specific host defense to pathogenic bacteria. Bifidobacterium spp. and Lactobacillus spp. belonging to the lactic acid bacteria group are the most commonly used in the prevention or treatment diseases, such as infections, cancer, inflammatory and autoimmune diseases. T-helper (Th) cells and T-regulatory (Treg) play central role in modulating immune responses. Dysregulation of the balance between two distinct cell subsets can cause recurrent spontaneous abortion. Probiotics have been shown to regulate inflammatory responses related to immune system through modulation of the anti-inflammatory cytokines such as TGF-β, IL-10 and also pro-

inflammatory cytokines, such as IL-6, IFN-γ. It seems that probiotic bacteria, could restore normal vagina microbiota and decrease inflammatory responses. Despite there are limited studies on the therapeutic impact of probiotics on these patients, the present study suggests several probiotics that improve immune responses in patients with RSA. Recent studies indicate that probiotics can restore tolerance and microbial balance, also modulate the important dysregulated different cells such as Th1/Th2/Th17/Treg and contributing to complaints of RSA.

Key words: *Recurrent Spontaneous Abortion, Immunomodulatory, Probiotic, T helper cells.*

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Effects of rheumatoid arthritis on infertility, pregnancy loss (review)

Nayebzade V, Yadegari M.
Anatomy and Cell Biology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: vajihenayebzade16@gmail.com

Introduction: Rheumatoid Arthritis (RA) is the most common type of autoimmune arthritis. The aim of this review was to study disease activity on fertility and time of pregnancy and possible risk of harmful effects for children born in mothers with RA.

Materials and Methods: For this study we used 12 peer reviewe articles published in Pubmed within 2015, which had most citation. Articles were relevant to RA in pregnancy and implantation rates. Variables were compared using one-way parametric analysis SPSS (version18).

Results: According to these articles, women with RA onset prior to conception had a slightly longer time of pregnancy and slight reduction in fertility. More than one-half of young women with RA achieve fewer biologic children than desire. immune regulation exhibited expression patterns that were differentially associated with pregnancy in the presence or absence of RA. This review support the hypothesis that the maternal immune system plays an important role during pregnancy, and also provide insight into other systemic changes that occur in the maternal transcriptome during pregnancy compared to the pre-pregnancy state.

Conclusion: Immune regulation exhibited complications that might occur in association with infertility and pregnancy in women with RA.

Key words: *Rheumatoid arthritis, Pregnancy, Fertilization, Children born.*

P-145
Autoimmune thyroid disease in pregnancy: A population-based study

Nazarpour S[1, 2], Ramezani Tehrani F[2], Simbar M[3], Tohidi M[4], Azizi F[5].
1. *Department of Midwifery, Islamic Azad University, Varamin-Pishva Branch, Tehran, Iran.*
2. *Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3. *Department of Reproductive Health and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
4. *Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
5. *Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: snazarpour@gmail.com

Introduction: Thyroid disorders are the second most common endocrinologic disorders found in pregnancy and Thyroid autoimmune disorders are relatively common in women. Observational studies demonstrated that thyroid autoantibodies even with normal thyroid function are associated with adverse pregnancy outcomes.

Materials and Methods: A total number of 2170 pregnant women were selected with population based cluster sampling in Shahid Beheshti University prenatal care centers. Data were collected by questionnaires that completed by trained interviewers. Serum thyroid stimulating hormone (TSH) and total thyroxine (TT4) were assayed by immunoradiometric and radioimmunoassay method, respectively. T3 uptake and thyroid peroxidase antibody (TPOAb) were measured by enzyme linked immunosorbent assay.TPOAb >50 IU/ml were considered TPOAb+.

Results: The results showed that 90.3% (n=1959) of pregnant women were TPOAb negative and 9.7% (n=211) were TPOAb positive. TPOAb+ pregnant women were included hyperthyroidism 0.09% (n=2), overt hypothyroidism 1.71% (n=37), subclinical hypothyroidism 4.93% (n=107), euthyroid 2.49% (n=54). Positive TPOAb in the women with a history of thyroid disorders was significantly more than those without this history (18.4% v.s 5.1%, $p \leq 0.0001$).

Conclusion: Thyroid autoimmune disorders are common in pregnancy and are correlated with previous history of thyroid disorders. Assessment of risk factors should be considered at screening program, in clinical practice. Further studies are needed to evaluate adverse effect of thyroid antibody positivity in euthyroid women on pregnancy outcomes.

Key words: *Thyroid, Autoimmunity, Thyroid antibodies, Prevalence.*

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The CD4+FoxP3+ CD52 high regulatory T cells in peripheral blood mononuclear cells and CD52 on sperm cells are down-regulated in unexplained infertility

Raeisi M[1], Kalantar K[1], Namavar Jahromi B[2], Gharesi-Fard B[3].
1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran.*
2. *Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*

Email: mohammad_reisi@yahoo.com

Introduction: Today, infertility is considered as a serious health care challenge. Several reasons are mentioned for infertility. Some of them are known and some are unknown or unexplained. As fetus is a semi allograft, thus immunological dysregulation could be considered for some pregnancy disorder. For instance, Preeclampsia, miscarriage and unexplained infertility. Treg cell is the main cells for regulation of immune system. Some evidences show that the defect in the function or decreasing number of these cells might also being the reason for infertility such as unexplained infertility.
Materials and Methods: 10 unexplained infertile couple and 5 fertile couple were enrolled in this study. Semen and whole blood was taken from all subjects. Flowcytometry done for checking the percent of CD52 Treg cells in PBMCs and levels of CD52 on the sperm cells. Elisa and Western blotting assay performed for evaluating of the levels of CD52 in the serum and semen of subjects. The Mann-Whitney test was used to compare differences between two independent groups.
Results: Intensity of CD52 in infertile men's sperm were decreased (433.4±160.5 vs 328.9±72). In addition, CD52 Treg cells also showed decreasing percent in women (88.40±10.45 vs 88.02±6.515) and men (93.30±4.712 vs 89.94±4.734) who were infertile compared to their controls. Finally, the levels of CD52 in the semen of infertile men decreased in the infertile men (158.2±84.47 vs 134.3±68.18). The Elisa result was confirmed by western blot assay.
Conclusion: Our obtained results suggest that low levels of this cells may be the reason for unexplained infertility because of their immune response are not suppress.
Key words: *Treg cells, CD52 molecules, Unexplained infertility.*

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The report of sever HPV wart lesions in the vagina of a 22 years old girl that received kidney transplantation and her healthy identical twins sister. Except of genetic and environment what is third factor in this Disease?

Athari SSh[1], Athari SM[2].
1. *Department of Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.*
2. *Department of Biology, Faculty of Basic Sciences, Maragheh University, Maragheh, Iran.*

Email: SS.Athari@gmail.com

Introduction: Kidney transplantation is the best replacement therapy in patients with end-stage renal disease. To prevent allograft rejection the immunosuppressive drugs should be used by patients that it increases the long-term risk of malignancy in renal transplant recipients. The immunosuppressed may predispose to development of HPV infection with potential to progress to cancer.
Materials and Methods: A single 22 yr old girl with minor thalassemia, due to renal failure in both kidney, received kidney transplantation from nonrelatives person. Then she began taking the immunosuppression drugs. Shortly wart lesions in cervix and vagina were appeared. The wart lesions have been very large.
Results: The patient has an identical twin sister and she was healthy and safe without renal failure, HPV or any diseases or problem. So monitoring of HPV infection in these patients should be doing before of any things. Furthermore screening urogenital neoplasms in kidney transplant recipients is necessary before and after grafting.
Conclusion: In these identical twins, why one of them is patient and another one is healthy? Here except of genetic and environment, the third and major factor is epigenetic and it has main role in many diseases so that need very attention.
Key words: *Kidney transplantation, Immunosuppression, Wart lesions, HPV, Healthy twin Sister, Third factor Epigenetic.*

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Evaluation of the cytokine TNF-α production by peripheral blood mononuclear cells of women with polycystic ovary syndrome by co-culture with breast cancer cell lines in compare with the healthy controls in vitro

Rezayat F[1], Hajiaghayi M[1], Mosaffa N[1], Ramezani Tehrani F[2].
1. *Department of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Tehran, Iran.*

Email: f_rezayat1991@yahoo.com

Introduction: TNF-α is an inflammatory mediator that can cause cancer due to its role in inflammation stability in body. The state of systemic this cytokine in patients of poly cystic ovary syndrome is the main reason for their immunological disorders. Possibility of malignancy for example breast cancer in these patients is under review.
Materials and Methods: 50 samples of isolated PBMNCs from peripheral blood samples (group of patients and healthy controls) were examined by ficoll density gradient centrifugation. MDA-MB-468 and MCF-7 tumor cell lines were incubated as two target cells and cultured adjacent to PBMCs in transwell system. At different time intervals (48 and 72 hours) after co-culture TNF-α concentrations were measured in supernatants by ELISA sandwich technique. Determination of CD3+CD8+ lymphocyte has rendered by flow cytometer.
Results: After 48 hr of incubation, TNF-α concentration was significantly more in group of patients in healthy controls. With continuing incubation up to 72 hr, TNF-α production was decreased by patients' mononuclear cells healthy controls.
Conclusion: Peripheral blood mononuclear cells of patients with PCOS are production of TNF-α up to 48 hr of incubation in vitro. The power of cell responses in patients with PCOS is reduced over time, outside of physiological condition.
Key words: *Polycystic ovarian syndrome, Breast cancer, Co-culture, TNF-α, CD3+CD8+ cells.*

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