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.*

2nd Congress of Reproductive Genetics

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.*

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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.*

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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.*