Oral Presentations

O-1

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

Yassini SM[1], Taghavi Shavazi N[2], Taghavi Shavazi M[3], Pourmovahed Z[1], Shiri Mohammad Abadi E[1].

1. *Research Center of Addiction and Behavioral Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Ali Ben Abi Taleb Medical College, Yazd Azad University, Yazd, Iran.*
3. *Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran.*

Email: yassiniard@yahoo.com

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

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

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

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

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

O-2

Macrophage migration inhibitory factor as a potential biomarker of endometriosis

Mahdian S[1,2], Shahhoseini M[1], Ramazanali F[2], Afsharian P[1], Ashrafi M[2], Aflatoonian R[2].

1. *Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: m.shahhoseini@royaninstitute.org; R.Aflatoonian@gmail.com

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

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

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

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

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

O-3

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

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

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

5. *Obstetrics and Gynecology Department, Iran University of Medical Sciences, Tehran, Iran.*
Email: R.Aflatoonian@gmail.com; femehra@yahoo.com

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

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

Zandieh Z[1, 2], Ashrafi M[1, 2], Pacey A[3], Aflatoonian R[4].
1. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2. *Department of Obstetrics and Gynecology, School of Medicine, Iran University of Medical Science, Tehran, Iran.*
3. *Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK.*
4. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: zandie.dvm@gmail.com

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

O-5

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

Abdollahi M[1], Salehnia M[2], Salehpour S[3].
1. *Department of Anatomical Sciences, Faculty of Medicine, Qom University of Medical Science, Qom, Iran.*
2. *Department of Anatomical Sciences, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.*
3. *Infertility and Reproductive Health Research Center (IRHRC), Ayatollah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: abdolahi_masume@yahoo.com

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

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

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

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

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

O-6

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

Dehghani MR[1], Zuffardi O[2].
1. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Molecular Medicine, University of Pavia, Pavia, Italy.*

Email: reza.dehghani@unipv.it

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

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

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

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

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

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

O-7

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

Esmaeili L[1], Salman Yazdi R[2], Esfandi H[3], Afraz K[4], Rajabian Naghdari M[5].

1. *US Department of Education, Royan Institute for Reproductive Biomedicine, Mashhad PNU University, Tehran, Iran.*
2. *Royan Institute for Reproductive Biomedicine, ACECR, Iran University, Tehran, Iran.*
3. *Royan Institute for Reproductive Biomedicine, Tehran University, Tehran, Iran.*
4. *Royan Institute for Reproductive Biomedicine, Oloom Tahghighat,Tehran, Iran.*
5. *Faculty of Sciences, PNU University, Mashhad, Iran.*

Email: lili_esmaili55@yahoo.com

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

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

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

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

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

O-8

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

Asadi E[1], Najafi A[1], Raeisi Dehkordi Z[2], Abolhasani F[1].

1. *Department of Anatomical Sciences, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
2. *Department of Midwifery, Shahrekord University of Medical Science, Shahrekord, Iran,*

Email: abolhasf@sina.tums.ac.ir

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

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

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

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

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

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

Shams Mofarahe Z[1], Ghaffari Novin M[1], Jafarabadi M[2], Salehnia M[3], Noroozian M[1], Ghorbanmehr N[4].
1. *Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
3. *Department of Anatomical Sciences, Tarbiat Modares University, Tehran, Iran.*
4. *Biotechnology Group, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.*

Email: zshmofarah@yahoo.com

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

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

Karimi-Zarchi M[1], Aflatoonian A[2], Karimzadeh MA[1], Mohsenzadeh M[2], Mousavi AS[1], Behtash N[1], Modarres-Gilani M[1].
1. *Department of Gynecology Oncology, Shahid Sadoughi University of Medical Science, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: drkarimi2001@yahoo.com

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

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

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

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

Halvaei I, Khalili MA, Safari S.
Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: ihalvaei@gmail.com

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

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

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

Abdi Sh[1], Salehnia M[1], Hosseinkhani S[2].
1. *Department of Anatomy, Tarbiat Modares University, Tehran, Iran.*
2. *Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: shabnam.abdi62@yahoo.com

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

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

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

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

O-13

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

Taheri F[1], Shirazi A[2], Nazari H[3], Ahmadi E[3].

1. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Veterinary Medicine Department, Shahrekord University, Shahrekord, Iran.*
3. *Institute of Animal Embryo Technology Department, Shahrekord University, Shahrekord, Iran.*

Email: taherifatemeh86@yahoo.com

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

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

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

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

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

O-14

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

Farajkhoda T[1], Najafi S[2], Bokaie M[2], Fotoohi Z[2], Moradi F[2].

1. *Research Center for Nursing and Midwifery care in Family Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: farajkhoda_t@yahoo.com

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

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

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

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

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

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

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

Shabani R[1, 2], Abolhasani F[3], Ashtari K[1, 4], Ashjari M[5], Izadyar F[6], Behnam B[1, 7], Asadi E[3], Koruji M[1, 2].

1. *Department of Anatomical Sciences, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2. *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.*
3. *Department of Anatomical Sciences, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
4. *Department of Medical Nanotechnology, Faculty of Advanced Technology in Medicine, Iran University of Medical Sciences, Tehran, Iran.*
5. *Department of Chemical Engineering, Faculty of Engineering, Kashan University, Kashan, Iran.*
6. *PrimeGen Biotech LLC, 213 Technology Drive, Irvine, CA 92618, USA.*
7. *Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Email: koruji@iums.ac.ir

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

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

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

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

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

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

Ahmadi F, Javam M, Haghighi H.
Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: dr.ahmadi1390@gmail.com

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

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

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

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

Faramarzi A, Khalili MA, Aflatoonian A, Solaimani M.
Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: faramarzi_90@yahoo.com

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

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

Allahveisi A[1], Ghaffari Novin M[2], Rezaei MJ[3], Nikkhoo B[4].
1. *Department of Anatomy, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
2. *Department of Anatomical Sciences and Biology, Faculty of Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3. *Department of Anatomy, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
4. *Department of Pathology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*

Email: allavaisie@gmail.com

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

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

Soleimani R[1, 2], Manolopoulos K[2], Fereidoni J[3], Kanayan S[4], Heytens E[1].
1. *Sol Scientifics Co. Gent, Belgium.*

2. *Kinderwunsch Zentrum, Offenbach, Germany.*
3. *Urmia Universitry of Medical Sciences, Urmia, Iran.*
4. *New Med Center, Yerevan, Armenia.*
Email: rezasoleimani@yahoo.com

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

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

Salehi Moghaddam Z, Bullock A, MacNeil Sh.
The Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield, UK.
Email: zoha.moghadam@gmail.com

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

O-21
Recurrent pregnancy loss causes and cures

Ghasemi N.
Recurrent Abortion Research Center, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: n479g@yahoo.co.uk; nghasemi479@gmail.com

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