Oral Presentations *(Alphabetic order)*

8th Yazd International Congress and Student Award in Reproductive Medicine

O-1
Sperm DNA damage have an effects on embryo aneuploidy in ICSI-CGH array cycles

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Background: There is clinical evidence to show that sperm DNA damage could be a marker of sperm quality and extensive data exist on the relationship between DNA damage and male fertility status. Sperm DNA fragmentation can be the most common reason for the transmission of anomalies of the father"s DNA to a child that seen in a high percentage of sperms in infertile men.
Objective: To investigate the effect of sperm DNA damage on embryo aneuploidy in ICSI-CGH array cycles.
Materials and Methods: In this study, 40 patients with recurrent implantation failure (RIF) were selected; they had at least 3 times of good quality embryo transfer with failure in the implantation. both women and men appeared to have no problem. All women were between 25-35 and stimulated with GnRH agonist and oocyte isolation was performed after follicles were picked up. Semen samples were also analyzed and DFI measured using TUNEL by flow cytometry. According to their TUNEL results, 2 groups were defined, 1: DFI >20% and 2: DFI <20%. Intra-cytoplasmic sperm injection was also performed and then the day 3 embryos were subjected to blastomere biopsy and evaluated by CGH array (Genomic Comparative Hybridization). Semen parameters, TAC, ROS and malondialdehyde (MDA) formation were analyzed between both groups also the correlation between embryo aneuploidy and semen parameters were evaluated.
Results: The results of this study showed that sperm with high DFI (DFI >20) significantly increased the number of the aneuploidy embryo than sperm with low DFI ($p<0.001$). Also, by increasing the DFI, the level of MDA significantly increased ($p<0.001$). The level of ROS and TAC were increased and decreased respectively but this was not significant by DFI ($p>0.001$).
Conclusion: These data indicate that sperm DNA damage have a significant effect on embryo chromosome aneuploidy. So, Embryo selection by aCGH should be considered in couples with high DNA fragmentation.
Key words: *Aneuploidy, DNA fragmentation, Malondialdehyde, TUNEL.*

O-2
The effect of neonatal maternal separation on the gelatinases activity of mouse ovarian follicles during in vitro culture

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Background: The critical role of the early life environment in the normal growth and development is identified.
Objective: This study investigated the effect of neonatal maternal separation on the gelatinases activity of mouse ovarian follicles during in vitro culture.
Materials and Methods: Female infants of NMRI mice immediately after their birth randomly allocated into two groups: maternal separation group (MS; separated from the dams for 6h per day from postnatal day 2 through 16) and control group (undisturbed over the 16 days). The litters were autopsied and isolated preantral follicles were in vitro cultured for 12 days. The developmental competence and gelatinase activity, the gene expressions of matrix metalloproteinases (MMP-2, 9) and their tissue inhibitors (TIMP-1, 2) were evaluated by zymogeraphy and real time qPCR respectively.
Results: There were significant differences between the two groups in the developmental competence, Follicles of MS groups showed a lower rate of growth, survival, antrum formation, ovulation and oocyte maturation than those of control group. The gelatinolytic activities were significantly lower in MS group compared with those of control groups. Furthermore, the gene expression levels of MMP2 and MMP9 in MS group compared with those of the control group significantly decreased. By contrast, the gene expression levels of TIMP-1 and TIMP-2 significantly increased in MS group compared with those of the control group.
Conclusion: MS as a stressor agent disrupt developmental competence of mouse ovarian follicles by changing in the gelatinase activity.
Key words: *Social stress, Ovarian follicle, Gelatinase activity.*

O-3
Effects of dietary approach to stop hypertension diet on androgens, antioxidant status, and body composition in overweight and obese women with polycystic ovary syndrome: A randomized controlled trial

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Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disease in reproductive age women.
Objective: The present study aimed to determine the effects of Dietary Approaches to Stop Hypertension (DASH) diet on reproductive hormones, plasma total antioxidant status and anthropometric indices in overweight and obese PCOS women.
Materials and Methods: In this randomized controlled clinical trial, 60 women with PCOS were randomly assigned to one of two diets with energy restriction: the DASH diet and a control diet. The DASH and control diets consisted of 50-55% carbohydrate, 15-20% protein and 25-30% total fat. The DASH diet was designed to be rich in vegetables, fruits, whole grains and low-fat dairy products, as well as low in saturated fats, cholesterol, refined grains and sweets. In the present study, the anthropometric indices, body composition, total testosterone, androstenedione, sex hormone binding globulin (SHBG), free androgen index and 2,2"-diphenyl-1-picryylhydrazyl (DPPH) scavenging activity were measured before and after 3 months.
Results: The consumption of DASH diet compared to the control diet was associated with a significant reduction in weight [-5.78 (1.91) kg vs. -4.34 (2.87) kg, p=0.032], body mass index (BMI) [-2.29 (0.15) kg m-2 vs. -1.69 (0.20) kg m-2 , p=0.02], fat mass [-3.23 (1.66) kg vs. -2.13 (1.26) kg, p=0.008] and serum androstenedione [-1.75 (1.39) ng mL-1 vs. -1.02 (0.72) ng mL-1, p=0.019]. Increased concentrations of SHBG [28.80 (21.71) versus 11.66 (18.82) nmol L-1, p=0.003) and DPPH scavenging activity [30.23% (19.09) vs. 12.97% (25.12) were also found in the DASH group.
Conclusion: The DASH diet could improve weight loss, BMI and fat mass. Furthermore, it could results in a significant reduction in serum androstenedione and a significant increase in antioxidant status and SHBG.
Key words: *Polycystic ovary syndrome, Dietary approach to stop hypertension, Androgen, Oxidative stress, Randomized controlled trial.*

O-4

Composite PU/SF hybrid tubular scaffolds for fabrication of engineered neovagina in-vivo explants in a 3D-culture perfusion bioreactor

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Background: Vaginal reconstruction is the choice medical approach in many congenital abnormalities, injuries, or cancers. Reconstructive techniques applying non-vaginal tissues can be associated with more complications. In special situations such as dilatation failure or large defects, surgical vaginoplasty is recommended. In this regard the main challenge is the lack of sufficient native tissue to reconstruct the organ.
Objective: Our aim is development of a tubular electrospun hybrid scaffold to fabricate engineered neovagina for these patients.
Materials and Methods: In this study, hybrid fibrous scaffolds of elastomeric polyurethane (PU) and silk fibroin (SF) with various mass ratios were fabricated by electrospinning. We expanded primary cultured human vaginal epithelial cells (HVECs) onto these scaffolds at a density of 1×10^5 cells/ml. Chemical and physical properties of scaffolds were evaluated using scanning electron microscopy (SEM), attenuated total reflectance Fourier transform infrared (ATR-FTIR), X-ray diffraction (XRD), contact angle measurement, biodegradation test and tensile strength analysis. The toxicity and biocompatibility of each scaffold was evaluated by the MTT assay using HVECs. Cell homing and proliferation was evaluated on the scaffolds by SEM and Hematoxylin & Eosin staining. Epithelial origin of the cell-seeded scaffolds characterized with RT-PCR analyses. PU/SF hybrid scaffolds were optimized to obtain the best imitator of normal vagina. Tubular structures of the optimized PU/SF nanofiber was fabricated by electrospinning with a novel designed rotating collector. The cell-seeded scaffolds were placed in the designed 3D perfusion bioreactor to prepare in vivo explants.
Results: SEM micrographs of electrospun scaffolds showed that the mass ratio of the PU/SF hybrid highly influenced the fibers morphology due to variations in conductivity and viscosity. By increasing the SF proportion, the fibers had lower diameters and higher uniformity. Surprisingly, ultrafine nanofibers and nanowebs had been widely distributed among the usual fibers. These nanowebs effectively increased the surface area and were favorable for cell attachment and spreading. The PU/SF scaffolds significantly promoted epithelial cell homing and proliferation compared to PU scaffold alone ($p<0.05$). RT-PCR analysis of growing cells on the tubular scaffolds confirmed the expression of epithelial cell surface markers such as cytokeratin19. Optimized PU/SF hybrid scaffold (60/40 ratio) with the most similar mechanical characteristics to normal vagina, was applied to produce tubular scaffolds. The cell-seeded tubular scaffolds, cultured in our designed perfusion bioreactor, had the same favorite properties as earlier 2D constructs. All of the results clarified that PU/SF 60/40 tubular scaffolds meet the required specifications for vaginal tissue engineering.

Conclusion: Results showed that the electrospun PU/SF 60/40 tubular scaffold possess proper biocompatibility and capability to promote vaginal tissue regeneration. 3D culture technology may be pursued further experiments in order to achieve engineered neovaginal tissues for the clinical applications.

Key words: *PU/SF tubular scaffolds, Electrospinning, 3D culture bioreactor, Tissue engineering, Neovagina*.

O-5

Gene expression profiling of cumulus cells isolated from MII oocytes of Iranian patients with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is a multifactorial, complex genetic, endocrine and metabolic disorder. It is clearly a heterogeneous syndrome, characterized by chronic anovulation, polycystic ovaries and biochemical and clinical manifestations of hyperandrogenism. PCOS is probably the most common cause of anovulatory infertility, associated with an increased risk of miscarriage after either spontaneous or assisted conception. However, with the recent technological advancements such as assisted reproductive technology (ART) that has been created to overcome the problem of infertility; these techniques are still expensive, and the success rate is low. Poor oocyte quality is the main cause of fertilization failure in ARTs. The selection of oocytes with the highest developmental potential for in vitro maturation (IVM) and in vitro fertilization (IVF) is currently based on morphological criteria, but it is generally acknowledged that its reliability requires further improvement. There is increasing evidence that communication between the oocyte and its surrounding cumulus cells (CCs) is vital for folliculogenesis and oocyte developmental competence acquisition. In this regard, it has been proposed that transcriptomic analysis of CCs will able us to predict oocyte developmental competence, as well as embryo and pregnancy outcomes during ART procedures (e.g., IVM and IVF).

Objective: Therefore, the aim of the present study was to use a non-invasive method in identifying potential oocyte developmental competence biomarkers for the selection of the best oocyte from women with PCOS; and the use of this oocyte to increase IVF success rates.

Materials and Methods: CCs from oocytes in metaphase II (MII) stage of PCOS and non-PCOS patients who underwent controlled ovarian stimulation were mechanically removed shortly before ICSI. Gene expression profiles were analyzed using the RNA-sequencing (RNA-seq) technology. Sequence reads were obtained from an Illumina HiSeq2500 platform and mapped onto the human genome (hg19) using TopHat aligner. The known gene annotation, functional annotation and gene-set enrichment analysis were performed using GO and KEGG databases on differentially expressed genes (DEGs) of CCs from PCOS and non-PCOS patients.

Results: The analysis of these RNA-seq data identified 59 genes that were differentially modulated in the two CC groups, including 42 genes that were up-regulated and 17 genes that were down-regulated in CC samples from patients with PCOS in comparison with patients without PCOS. Some of these genes were identified to be involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation, oocyte maturation and regulation of luteinizing hormone.

Conclusion: Gene expression profiles showed clear difference between CCs from non-PCOS and those from PCOS patients. This study identified candidate genes involved in cell cycle progression and differentiation and also oocyte maturation that may influence the function of granulosa cells in PCOS patients. Our results may be clinically important as they offer a new potential strategy for competent oocyte/embryo selection in PCOS patients.

Key words: *Polycystic ovary syndrome, Cumulus cells, Gene expression, RNA-sequencing, Non-invasive biomarkers.*

O-6

KHDC3L frame-shift mutation causes recurrent pregnancy loss and infertility in an Iranian family

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Background: Recurrent pregnancy loss (RPL) is a common infertility-related disease affecting about 1-3% of women worldwide. The parental and embryo different conditions have been associated with RPL. However, it has been estimated that approximately half of cases remains unexplained.

Objective: In recent years, next generation sequencing technology, acts as a robust tool to discovery of causal mutations in hereditary diseases such as RPL.

Materials and Methods: In this study, we recruited an Iranian family with RPL history by whole-exome

sequencing approach. Then, the effect of selected candidate pathogenic variant was confirmed using Sanger sequencing method in family members.

Results: Clinical investigations such as thrombophilias, uterine anatomy, hormonal/endocrine disorders, immunologic factors, infections and karyotyping in proband were normal. *KHDC3L* mutation was identified in 50% and 100% analyzed subjects with RPL and hydatidiform mole (HM) history, respectively.

Conclusion: In conclusion, the results, for the first time, indicated that the detected variant in *KHDC3L* gene is involved not only in the HM but can also contribute to RPL that is in contrast with previous studies. Therefore, our findings confirm that WES is a useful alternative approach to Sanger sequencing to reach a genetic diagnosis in patients.

Key words: *Recurrent pregnancy loss, Whole exome sequencing, KHDC3L, Hydatidiform mole.*

O-7

Evaluation of novel mouse specific germ cell gene expression in embryonic stem cell- derived germ cell-like cells in vitro with retinoic acid treatment

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Background: Defects in natural and complete meiosis are one of the obstacles in achieving functional gametes. Retinoic acid (RA) induced intracellular signals also play an important role in germ cell differentiation.

Objective: The goal of the present study was to evaluate an in vitro differentiation model of mouse embryonic stem cells into germ cells, using RA.

Materials and Methods: There are three groups in this experimental study. 1-ESCs differentiated using EB method for 7 days then EB aggregation singled and cultured with RA for 7 days as (+RA) 2- EB aggregation in day 7 singled and cultured without RA for 7 days as control group (-RA) 3- EB aggregation in day 7 of culture as (EB7). We designed a study to induce differentiation of Oct4-GFP (expression of Green Fluorescent Protein of oct4) embryonic stem cells (ESCs) by embryoid body (EB) culture system into germ cells using RA and evaluated the expression level of (Fkbp6, Mov10l1, 4930432K21Rik, Tex13) in differentiated cells. The expression levels of 4 GC-related genes, Oct4, Mvh, Scp3 and Stra8 was determined by q-RT-PCR. Immunostaining and Flowcytometry used as additional test to confirm q-RT-PCR findings.

Results: Significant increase occurred in expression of meiotic markers and specific genes Fkbp6 (p=0.00), Mov10l1 (p=0.01) and Tex13 (p=0.00) in ESCs treated with Retinoic Acid (+RA) compared to the controls (-RA). Oct4 expression was decreased in all studied groups. The expression levels of 4930432K21Rik, Mvh, Stra8 and Scp3 in the +RA group was higher than that of the -RA group. Flowcytometry analysis showed that mean number of Mvh-positive cells in the +RA group was greater as compared with ESCs, -RA and EB7 groups (p=0.00). Immunofluorescence staining showed that higher Mvh expression staining was observed in the +RA group, compared to that of the -RA group, which confirmed the data from the q-RT-PCR. The cells characterizing GCLCs with round nuclei are shown by Hoechst nuclei counterstaining.

Conclusion: These 4 specific germ cells genes are expressed in the testis, but not the ovary, which indicates their role in the development of male gametes. According to the findings, down regulation of Oct4 as a pluripotency factor as well as the expression of meiosis markers this hypothesis is raised that ESCs are differentiated by RA, and have been introduced into the zygote / pachytene of first meiosis as germ cell-like cells. So induction of mESCs by RA may cause differentiation towards GCLCs. Improving in vitro germ cell differentiation with high efficiency may simplify the generation of mature gametes for understanding of biology of gametogenesis.

Key words: *Embryonic stem cells, Primordial germ cells, Retinoic acid, Embryoid body.*

O-8

Carob promotes spermatogenesis in infertile mice model via inducing the self-renewal process in spermatogonial stem cells

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Background: Herbal medicine, also called botanical medicine or phytomedicine, refers to using a plant's seeds, leaves and others for medicinal purposes. Herbs are widely used today, in teas, vitamins and natural supplements. While the benefits of herbal medicine are vast, it is important to understand the basis of herbal medicine. Ceratonia silique l.(Carob) extract is being traditionally used for male infertility treatments. However, there is not sufficient scientific basis for using this herb and the mechanism underlying its function yet to be understand.

Objective: Herein, we evaluated the "Carob extract" in infertile mice model and uncovered the mechanism underlying its function.

Materials and Methods: In this study, 160 male mice (mean age: 6-8 wk) were divided into five groups as follow: intact, vehicle, positive control and four experiment groups that received different doses of Carob extract by oral gavage for 35 days. For creation sterile model, we injected a single dose of 45 mg/kg bw busulfan into the peritoneum. The positive control was administered 25 mg/kg bw of Clomiphene citrate and Carob groups received oral gavage doses: 75, 150, 300 and 600 mg/kg bw. The sperm parameters, testicular

histopathology, DNA content, ROS level as well as gene expression analysis for spermatogenesis-related genes were investigated after 35 days of the extract.

Results: Our results demonstrated that Carob administration significantly increased sperm count and motility in a dose dependent manner. Moreover, this extract significantly reduced the DNA fragmentation and ROS level in sperm cell population. Histological analysis revealed increased spermatogenesis following carob extract gavage. Gene expression analysis confirmed significant increase in the expression levels of spermatogonial stem cells (SSCs) self-renewal genes that including Plzf, Gfr-1, Bcl-6b, Utf-1 as well as differentiating-related genes that consist of Dazl, C-kit, Ngn3, Stra8.

Conclusion: We have shown that, carob as a natural compound have the ability to induce spermatogenesis in infertile mice model. Our results suggest that Carob promotes both self-renewal of SSCs and differentiation of spermatogonia that are necessary to support the spermatogenesis. Our primary data suggest the Carob as an effective herbal medicine that can be used for non-obstructive azoospermia men. However, more investigation needs to demystify its function before applying it in clinic.

Key words: *Azoospermia, Herbal medicine, Spermatogenesis, SSC.*

O-9

Human embryonic stem cells' differentiation into cardiomyocytes: TLRs expression during process

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Background: The trans-membrane receptor family of Toll-like receptors (TLRs) is expressed in immune cells and cardiac muscle. To date, several TLRs (numbered 1-11) have been identified in humans. TLRs may play a role in initiating early inflammatory and functional responses to danger signals arising from ischemia-reperfusion and inflammatory stimuli. Human embryonic stem cells (hESCs) have the potential to provide an unlimited source of cardiomyocytes, which are invaluable resources for drug or toxicology screening, medical research, and cell therapy.

Objective: Human embryonic stem cells (hESCs) have the potential to provide an unlimited source of cardiomyocytes, which are invaluable resources for drug or toxicology screening, medical research, and cell therapy.

Materials and Methods: Royan H5 and Royan H6 hESC lines were used in this study. Suspension culture of hESCs was performed according to a recently published protocol. Differentiation of the cells into cardiomyocytes in suspension was performed according to the Laflamme et al. protocol with some modifications. In this study, expression of TLR2, TLR3, TLR4, TLR5 and TLR9 was evaluated by RT-PCR and Q-PCR during cardiomyocyte differentiation on day 8, 14 and 25 (mature cardiomyocyte).

Results: According to Q-PCR data, TLR9 expression has increased and TLR5 expression has decreased in linear pattern during cardiomyocyte differentiation. Expression pattern of TLR2 and TLR4 was same in sigmoid shape. In both of them, expression level was lowest in mature cardiomyosyte. Also, about TLR3 expression, our data was shown sigmoid shape but the lowest expression related to 8th day after differentiation.

Conclusion: The goal of this study was to investigate the ability TLR expression in cardiomyocyte differentiation from human embryonic stem cells. According to results, all of TLRs in this study have been expressed in cardiomyocyte in variable level. TLRs expression related to inflammatory responses, ischemia and contractility. On the other hand, myocardial infarction and all of myocardial ischemia diseases have inflammatory reasons. Hence, evaluation of TLRs expression during cardiomyocyte differentiation is indispensable in cardiac cell based therapy.

Key words: *Cardiomyocyte, Differentiation, Human embryonic stem cells, Toll like receptors.*

O-10

Duphaston for preventing premature luteinizing hormone surges in women with polycystic ovarian syndrome undergoing controlled ovarian hyper stimulation: A randomized clinical trial

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Background: Polycystic ovarian syndrome, is considered a prevalent endocrine disorder. IVF (in vitro fertilization) is the major therapy. The use of new ways of improving clinical results is yet promptly required. It has been proved that progesterone (P) prevents pulsatile LH and GnRH secretion seriously and inhibits the E2-caused effects of the positive feedback throughout the luteal stage via reducing the frequency of the GnRH pulse.

Objective: An RCT was devised to assess the cycle features and endocrinological characteristics of individuals using gonadotrophin accompanied by receiving dydrogesterone co-treatment, and to compare pregnancy results in FET (first frozen embryo transfer) cycles with the antagonist-GnRh protocol considered as the control.

Materials and Methods: A total of 120 individuals with PCOS, who volunteered for ART, got included in the research. The patients were classified into two groups. In PPOS group the patents received 20 mg dydrogesterone orally since the second day of the cycle. All patients in controlled group received Antagonist protocol.

Results: From total 120 patients who had including criteria, 60 people were selected for each group randomly. No. of MII oocyte, maturity rate, No. of 2 pronuclei (2PN) and serum E2 levels on trigger day were

lower in PPOS group ($p<0.05$). Serum LH level on trigger day in PPOS group was higher than antagonist group (5.29 vs 3.79; $p<0.05$).Although there wasn't sever OHSS in any patient, mild and moderate OHSS was less in PPOS group (36.5% vs. 68.3%; $p<0.05$). Chemical and clinical pregnancy were more in antagonist group, but it was not statistically significant ($p>0.05$).

Conclusion: Our study fails to demonstrate that PPOS has the potential to improve chemical and clinical pregnancy rate of the infertile women with PCOS.

Key words: *Progesterone, Polycystic ovarian syndrome, Controlled ovarian stimulation, Frozen thawed embryo transfer, Pregnancy rate.*

O-11
An investigation of the cell stemness of the human ovarian derived epithelial-like cells

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Background: This is an old dogma that mammals are born with a fixed number of primordial follicles, each of which encloses an oocyte arrested at the diplotene step of meiotic prophase, and the primordial follicle pool cannot be replenished after birth. Johnson et al (2004) have shown that juvenile and adult mouse ovaries possess mitotically active germ cells and have an ability to produce new oocytes and follicles from germ line stem cells (GSCs) present in the ovarian surface epithelium after their destruction that can be fertilized to produce viable offspring.

Objective: Here, the stemness of the epithelial-like cells derived from human ovaries was assessed.

Materials and Methods: Following fully consent approval small samples were collected by gently scraping of human ovaries. Samples transferred to the lab and human epithelial-like cells were isolated and cultured after rinsing and centrifugation. While initial colonies were formed and cells started to expand their gene expression profile was investigated using PCR for OCT4, NANOG, SOX2, SOX17, DAZL, VASA, SCP3, GDF9, and FSHR. Moreover, IF was applied for SSEA4, C-KIT, VASA, FIBRONECTIN and VIMENTIN.

Results: Epithelial-like cells were proliferated while showing colony formation patterns in the culture. Cells from the initial cultures (day4-5) only expressed VASA by PCR. IF data revealed the expression of SSEA4 (stem cell marker) and VASA (germ cell marker) by some populations of the cells.

Conclusion: Small numbers of the cells from the initial cultures of the epithelial-like cells from human ovaries expressed VASA and SSEA4 which indicates there might be some cells with germ cell origin and stem cell features. However, further investigations are required with longer cultures.

Key word: *Epithelial-like cells, Ovarian stem cells, Germ-line stem cells, Stemness.*

O-12
Women's satisfaction with modern contraceptive methods utilization and associated factors in Bishoftu, Ethiopia, 2016

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Background: Client satisfaction is the base for effective and efficient utilization of modern contraceptive methods in all setting to reduce maternal and child mortality. However, there were limited evidences in factors affecting client satisfaction with modern contraceptive methods utilization in the study area.

Objective: This study aimed to identified factors affecting women's satisfaction with modern contraceptive methods utilization among reproductive age women.

Materials and Methods: Institutional based cross sectional study was conducted from February 1 to March 31/2016 on 422 individual who randomly were selected from family planning service of Bishoftu town governmental health institutions. Simple random sampling technique was employed to select the study participants. A pre-tested structured questionnaire was used to collect the data. Bivariate and multivariate logistic regressions were used to identify factors associated with client satisfaction on modern family planning methods utilization. Adjusted odds ratio with 95% confidence interval and $p<0.05$ were computed to determine level of significance.

Results: Women's satisfaction with modern contraceptive methods utilization was found to be 94.7%. Educational status of women [AOR=5.74 (95% CI: 1.271-25.884)], employment status of women [AOR=2.86 (95%CI: 1.042-7.840)], marital status [AOR=8.74 (95% CI: 1.117-68.348)], number of visit [AOR=3.33 (95% CI: 1.077-10.280)], had communication with husband [AOR=2.37 (95% CI: 1.853-6.577)] and difficulty of understanding health care provider`s counseling [AOR=0.09, (95% CI: 0.02-0.41)] were factors significantly associated with client satisfaction on modern contraceptive methods utilization.

Conclusion: Women's satisfaction with modern contraceptive methods utilization was high. Increasing the number of health institution visit and encourage education might improve and maintain women's satisfaction.

Key words: *Ethiopia, Contraceptive utilization, Client satisfaction.*

O-13

Detection of hyaluric acid synthesis 2 (has2) and gremlin 1 (grem1) gene expressions in human cumulus cells of IVF patients as oocyte quality indicator

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Background: Nowadays, the criteria for oocyte selection is based on morphological criteria however, it needs further improvement to select the best embryo quality. The gene expression in the cumulus cell plays important role in signaling for follicular development as well as for oocyte quality.

Objective: The aim of this study is to investigate the present of HAS2 and GREM1 gene expression in the cumulus cells that can become a useful marker for oocyte quality.

Materials and Methods: A case study was performed on cumulus cells derived from four different patients that undergo assisted reproductive technique treatment. Cumulus cells were isolated from 4 IVF patients who have different pathological conditions, oocyte and embryo grading. The expressions of HAS2 and GREM1 were analyzed by using reverse transcriptase polymerase chain reaction (RT-PCR). The products of the PCR were then being quantified and statistical analysis have been done to test the significant of the data.

Results: The results showed that HAS2 and GREM1 were expressed differently in each patient. Those genes have been detected in patient 2, 3 and 4 with grade 3 oocytes, whereas, the genes were absent in patient 1 with grade 4 oocytes. The embryo grades and pathological conditions have shown no influence in those genes expression. This showed that the genes expression influenced the oocyte quality.

Conclusion: Hence, the measurement of HAS2 and GREM1 expressions in cumulus cells would possibly useful as a tool for selecting competence oocytes with greater chances to be fertilized in assisted reproductive technique.

Key words: *Cumulus cells, HAS2, GREM1, RT-PCR, oocyte quality.*

O-14

Optimizing the cell seeding protocol to human decellularized ovarian scaffold: application of dynamic system for bio-engineering

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Background: Decellularized tissue scaffolds provide an extracellular matrix controlling the stem cells differentiation toward specific lineages. Mesenchymal stem cells application for artificial ovary production may enhance ex vivo functions of the ovary. On the other hand, the scaffold needs interaction and integration with cells.

Objective: Therefore, develop of ovarian engineered constructs (OVECs) requires the use of best method for well seeding of the cells into the ovarian and the other type of scaffolds. The main goal of the present study was to develop an optimized culture system for the efficient seeding of peritoneum mesenchymal stem cells (PMSCs) into human decellularized ovarian scaffold.

Materials and Methods: In experimental study, three methods were used for cellular seeding including rotational (spinner flask) and static (conventional and injection) seeding cultures. OVECs were evaluated with Hematoxylin and Eosin staining and viability analyses for the seeded PMSCs. Then, immunohistochemistry analysis was performed in best method of cellular seeding for primordial germ cell-like cells, mesenchymal stem cells and proliferation markers. Also, stereology analysis was made for the number of penetrated cells in depth of the OVECs.

Results: Our results revealed that rotational seeding increases the permeability of PMSCs into the scaffold and survival rate of the seeded PMSCs comparing to the other methods, at the same time. On the other hand, the rotational seeded PMSCs had well capability of proliferation with ki67 expression and differentiation to ovarian specific cells with expression of primordial germ cell line markers without mesenchymal stem cells markers production. Furthermore, stereology showed a well distribution of PMSCs along the outer surfaces of the OVECs with further distribution at the central part of scaffold. Average total cell values were determined 2142187 cells/mm3 on each OVEC.

Conclusion: The rotational seeding method is more appropriate approach for cell seeding into decellularized tissues compared to static seeding.

Key words: *Peritoneum mesenchymal stem cells, Rotational seeding, Tissue engineered ovary, Stereology.*

O-15

Sperm DFI affects growth factors expression: as a consequence of fallopian tube and spermatozoa interaction

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Background: The changes that occur in the female reproductive tract during the menstrual cycle and in early pregnancy, in preparation for embryo implantation and subsequent placental and fetal development, have long attracted the interest of reproductive biologists. The early embryo expresses growth factors and receptors that are, in general, temporally expressed. In addition, the oviduct and uterine also synthesize growth factors and cytokines. A growth factor is naturally occurring substance capable of stimulating cellular growth, proliferation, healing, and cellular differentiating. Growth factors are important for regulating a variety of cellular processes. As with many other tissues, different growth factors like the CSFs, VEGF, LIF, MSTN and BMPs seem to have major effect on the female reproductive tract growth particularly fallopian tubes. Fallopian tube is a part of female reproductive tract in which fertilization, sperm preservation, capacitation and so on usually occurs.
Objective: The aim of current research is to investigate the influence DNA fragmentation index (DFI) of sperm on the expression of different growth factors.
Materials and Methods: Sperm samples from 10 donors with normal features were collected and classified to two groups of normal and abnormal DFI. The extent of sperm DNA fragmentation, which measured by the TUNEL assay. The third group was fallopian tube cells without sperm. Finally, different sperms were co-incubated with fallopian tube cells for 24h. PCR array was performed to evaluate of growth factor genes expression profiling. We analyzed gene expression of CSF1, CSF 2, CSF 3, VEGF, LIF, MSTN, LIF, BMP2, BMP4, BMP6 and BMP7 among growth factor genes. In addition, this data was validated by q-PCR.
Results: The results of the data analysis indicated that the fallopian tube expresses the growth factors. It was also observed that the expression of some growth factors in the vicinity of sperm significantly changes. The data shows that the expression of growth factors was not significantly different between normal and abnormal sperm groups. However, their expression was lower than the control group. In contrast with these genes, BMPs had higher expression in the sperm groups.
Conclusion: The results of this study indicate that the fallopian tube reacts to sperm presence, and by changing the expression of growth factors can be effective in maintaining the function of sperm and protecting the fallopian tube. Sperm with abnormal DFI can causes excessive Stimulation of the fallopian tube and different expression of these growth factors compared to normal DFI may lead to changes in reproductive activity. It seems that the findings of this research can be considered in the process of assisted reproductive technic and bring better results.
Key words: *Growth Factor, Sperm, DNA Fragmentation Index (DFI), Fallopian Tube, PCR Array.*

O-16
Determination of the prevalence of cytomegalo virus, papilloma virus and herpes simplex virus in infertile men referring to IVF center, Afzalipour Hospital in Kerman in 2017

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Background: Infertility is a major problem of modern medicine as it affects almost 20% of reproductive age couples. In 40-50% of cases, infertility is associated with men. Most male genital tract infections may induce infertility, Chronic viral infections can infect sperm and are one of the risk factors for male infertility. Viral infections can lead to male infertility by directly affecting the genital tract cells and indirect stimulating immunological responses. Herpes simplex virus, papilloma and cytomegalovirus can cause male genital tract infections without symptoms in a long period of time. There was no study on the prevalence of viral infections in infertile men and the association of male infertility with viral infections in Kerman province.
Objective: We aimed to investigate the prevalence of viral infections (HCMV, HPV, and HSV1,2) in infertile men referred to the IVF Center, Afzalipour Hospital, in order to study correlation between infertility and viral infection in men.
Materials and Methods: In a case-control study, at first, the samples were analyzed according to WHO guidelines. Then, based on the results of seminal fluid analysis, the samples were divided into two groups the case (100 samples) with an average age of 34.84 ± 5 and control group (100 samples) with an average age of 34.24±4.69, respectively. All samples were examined for the presence of HCMV DNA, HPV DNA, and HSV 1,2 DNA with Real Time PCR method. Data analysis was performed using SPSS version 19.
Results: Based on the results of this study, the prevalence of HCMV infection in the seminal fluid of the case group was 23 (23%), which was significantly higher than the control group 7 (7%) (p=0.002). Also, the prevalence of HSV1.2 infection in infertile men was 4 (4%), which was significantly higher than fertile men (0) (p=0.04). There was no significant difference in the number of cases in the case and control groups in relation to the prevalence of HPV infection (p=0.52).
Conclusion: According to our results, due to the high prevalence of HCMV and HSV1.2 infection in infertile men to fertile men there is a potential for a reduction in the count and motility of sperm and infertility caused by these viruses.
Key words: *IVF, HCMV, HSV 1, 2. HPV, Infertility.*

O-17

Impact of vitrification on human oocytes before and after in vitro maturation (IVM): A systematic review and meta-analysis

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Background: Combination of in vitro maturation (IVM) and cryopreservation offers new opportunities for women with contraindication in ovarian stimulation, and females who desire to postpone the childbearing due to different problems. There are still controversies regarding IVM procedure and its impact on oocytes fertilization capability.

Objective: The aim of this systematic review and meta-analysis was to evaluate the impact of vitrification on human oocytes during IVM procedure.

Materials and Methods: A systematic review with meta-analysis was undertaken. Main search terms were those related key words. We searched Medline, Embase, Scopus and ISI web of science to detect English-language studies. The original articles which studied ART outcomes after vitrification of MII or GV oocytes before or after IVM were included. Studies that compared the combination of vitrification and IVM with fresh oocytes were also included. Exclusion criteria were animal trials and the studies that performed cryopreservation using slow-freeze method. The protocol of the review has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) by registration number of CRD42017054372.

Results: 2476 articles were screened and after duplicates removing together with the application of inclusion and exclusion criteria, 14 studies assessed for eligibility. Finally, 5 studies included for analysis. All studies compared laboratory outcomes between oocytes that vitrified at the GV stage and those which firstly matured in vitro, and then vitrified. Meta-analysis showed that vitrification of oocytes at GV stage had a negative impact on maturation rate (RR=1.28, 95% CI: 0.96-1.70); but not on cleavage rate (RR=1.07, 95% CI: 0.70-1.64); fertilization rate (RR=0.99, 95% CI: 0.85-1.14) and survival rate (RR=1.01, 95% CI: 0.96-1.06).

Conclusion: In general, based on our results, oocyte vitrification decreases the maturation rate. In addition, survival, fertilization as well as cleavage rates did not significantly differ between the oocytes vitrified before IVM versus oocytes vitrified after IVM. However, there was no significant difference in oocyte maturation rate regarding vitrification before and after IVM.

Key words: *Human oocyte Vitrification, in vitro maturation, Cryopreservation, Fertility Preservation.*

O-18

Different sperm DFI induced fallopian tube response by chemokines

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Background: The human fallopian tube is an active assembly that undergoes the variation of chemokines. Chemokines are one of the major compartments of immune system. Sperm and fallopian tube interaction in the female reproductive tract has an important role in fertilization, early embryo development, implantation and pregnancy. It was showed that the chemokines including CCLs, CXCLs, CX3CL1 and PPBP have relevance in ovulation, sperm capacitation and fertilization.

Objective: To understand further the basis of maternal communication with spermatozoa and the role that innate immune system plays in this interaction, we evaluated the expression of chemokine in fallopian tube cells in the presence of spermatozoa with normal and abnormal DFI compared with control group.

Materials and Methods: In this investigation, sperm samples from 20 donors with normal features were collected and classified to two groups of normal and abnormal DFI. The control group was fallopian cells without sperm. Sperms were co-incubated with fallopian cells for 24h. Afterwards, the level of chemokines mRNA expression was compared using Real time PCR Array. The mRNA of chemokines was analyzed in 3 groups (control, normal DFI, abnormal DFI, n=10/per group).
We analyzed gene expression of CX3CL1, CXCL10, CXCL11, CXCL13, CCL3, CCL8, CCL11, CCL20, CCL24 and PPBP among chemokines genes. This data was confirmed by q-PCR.

Results: Data analysis demonstrate that the expression of chemokines in the presence of sperm decreased compared to control group. Our findings are compatible with previous studies which show that chemokines play fundamental role in the interaction between sperm and female reproductive tract. Generally, the expression of CCLs and CXCLs was lower in the sperm groups than the control.

Conclusion: It seems that abnormal sperm DFI is established as a pathogen which leads the decreasing of chemokines in fallopian tubes. Therefore, it can be concluded that chemokines are essential for sperm and fallopian tube interaction. This communication prepared safe environment for important events in fallopian such as sperm preservation, fertilization and so on. Further investigation should be direct on the effect of chemokines rule to clarify optimum fertilization.

Key words: *Chemokine, Fallopian tube, Sperm, DFI, Fertilization, PCR Array.*

O-19

Reactive oxygen species by fungicide mancozeb in Sertoli- germ cell co- culture

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Background: Oxidative stress has been extensively studied as a cause of male infertility and excessive levels of reactive oxygen species (ROS) coupled with a deficiency in antioxidants can lead to it. ROS are derived either from internal sources in the body or from external sources such as exposures to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Exposure to pesticides affects the reproductive system. They can produce free radicals and change antioxidant capacity or free radicals by inducing oxidative stress. Mancozeb is a typical fungicide that has been shown to produce adverse effects and toxicological manifestations in fertilization, damage to liver, kidney, central nervous system and chromosomes of bone marrow cells in mice.
Objective: In the present study, we evaluated the potential of mancozeb oxidative stress induction in Sertoli-germ cell co-culture.
Materials and Methods: In this study, ROS generation was measured using DCFH-DA. Testes from mic were dissected, de-capsulated and teased in 10 ml PBS. Cells were isolated from the tubules after collagenase treatment and trypsin treatment at 32^{o}C in a water bath with gentle shaking in the presence of DNase I. Cells were grown in complete DMEM/F12 containing 15% FBS in a humidified incubator of 5% CO2 at 37^{o}C. Cells were loaded with 25 µM DCF for 60 min, then treated with the appropriate concentrations of mancozeb (1, 2.5, 3.5 µM) for 3 hr. To investigate induction of oxidative stress, we also used an antioxidant N-acetylcysteine (NAC). The generation of intracellular ROS was quantitatively monitored in a microplate reader.
Results: In the presence of 2.5 and 3.5 µM mancozeb, ROS levels was increased in Sertoli-germ cell co-culture.
Conclusion: Our results confirm that mancozeb can mediate cellular toxicity through reactive oxygen species generation which can play a role as a mediator of apoptotic cell.
Key words: *Fungicide, Mancozeb, ROS, Sertoli-germ cell co-culture.*

O-20

Effects of canthaxanthin on sperm parameters during human sperm freeze-thaw process

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Background: Sperm cryopreservation has detrimental effects on sperm parameters. In order to reduce these adverse effects, various strategies have been proposed including the use of antioxidant during cryopreservation.
Objective: The main goal was to evaluate the effect of canthaxanthin on preservation of sperm motility, viability, morphology, acrosome reaction, sperm chromatin packaging, and DNA integrity during freeze-thaw process.
Materials and Methods: Twenty-five normozoospermic semen samples were collected. After initial evaluation, samples were processed by direct swim-up. Before cryopreservation, all sperm parameters including motility, viability (eosin-nigrosin), morphology (Papanicolau), chromatin packaging (aniline blue and toluidine blue) and DNA denaturation (acridine orange) and fragmentation (sperm chromatin dispersion test) were evaluated. Then, each sample was divided into five groups including 0, 0.1µM, 1µM, 10µM and 25µM of canthaxanthin. Samples were frozen by rapid freezing technique. The spermatozoa were thawed and all sperm parameters were re-examined.
Results: All sperm parameters after freeze-thaw process significantly decreased compared to before freezing. But, despite this reduction, antioxidant supplementation has been able to improve sperm parameters. 25µM group could significantly improve the progressive and total motility, viability, normal morphology, chromatin packaging, acrosome integrity and DNA denaturation and fragmentation. 10µM group significantly improved total motility, viability, normal morphology, chromatin packaging, acrosome integrity and DNA denaturation and fragmentation. Whereas, in 1µM group there were significantly differences only in improvement of acrosome integrity, chromatin packaging (toluidine blue) and DNA denaturation and fragmentation. But, in 0.1µM group, there was no significant differences in any of measured parameters.
Conclusion: It seems canthaxanthin can protect the sperm motility, viability, acrosome integrity, normal morphology, and DNA integrity during cryopreservation.
Key words: *Cryopreservation, Sperm, Antioxidant, Canthaxanthin.*

O-21

Assessment of oxytocin level, glucose metabolism components and cutoff values for oxytocin and anti-mullerian hormone in infertile PCOS women

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Background: Polycystic ovary syndrome (PCOS) is a common problem among women with infertility or subfertility. PCOS is associated with multiple complex hormonal and metabolic changes.
Objective: Comparing oxytocin level and some other parameters between infertile women with or without PCOS, to evaluate the correlation between oxytocin with anti-mullerian hormone (AMH), Body Mass Index (BMI) and insulin resistance (IR).
Materials and Methods: This cross-sectional study was performed on 80 PCOS and 81 non-PCOS women as the control group. Oxytocin, various hormones, Oral glucose tolerance test (OGTT) and Homeostatic model assessment of insulin resistance (HOMA-IR) were compared between two groups. Correlations between parameters were assessed by the spearman's rank correlation coefficient. Cutoff values for oxytocin and AMH in PCOS were calculated by the ROC-Curve and DeLong method.
Results: The mean oxytocin level was statistically lower in the case group ($p \leq 0.001$). The mean BMI, AMH, HOMA-IR, fasting insulin and insulin 2-hr after 75-g glucose were significantly higher in the PCOS group. Oxytocin was negatively correlated to AMH when evaluated for all participants or only among controls. Moreover, oxytocin was negatively correlated to HOMA-IR among all participants. However, the relationship between oxytocin and BMI was not statistically significant. The calculated cutoff value for oxytocin was 125 ng/L and for AMH was 3.6 ng/mL in the PCOS group.
Conclusion: The mean oxytocin level in the PCOS infertile women was lower than non-PCOS women. Oxytocin showed a significant reverse correlation with AMH and HOMA-IR.
Key words: *Anti-mullerian hormone, Oral glucose tolerance test (OGTT), Oxytocin, Polycystic ovary syndrome.*

O-22
Evaluation of decellularized ovarian scaffolds by triton, ammonium hydroxide and SDS

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Background: Decellularization is a novel technique in regenerative medicine. Recently decellularized ovary introduced as a scaffold in the field of human fertility preservation. So the aim of the present study was producing a decellularized ovarian scaffold for fertility preservation approaches.
Objective: The aim of the present study was the evaluation ovarian cortex decellularization by Triton, Ammonium hydroxide and SDS.
Materials and Methods: In the present study 2 mm pieces of bovine and human ovarian cortex were prepared. In the SDS group, ovarian cortex was decellularized with 0.1% SDS for 24 hr, in the SDS-Triton-ammonium group these pieces were decellularized with 0.5% SDS for 2 hr, 1% Triton and 0.1% Ammonium hydroxide for 22 hr and in SDS-Triton group pieces were decellularized with 0.5% SDS for 3 hr and 1% Triton for 9 h. Human ovarian cortex was decellularized in three steps. First, it was treated with 0.1% SDS for 24 hr then according to freezing and thawing, it was treated with 0.2% SDS for 10 hours. After decellularization, all of the scaffolds were washed with deionized water for 24 hr. The intact ovarian cortex was used as a control group. H&E and DAPI staining were applied to prove decellularization. Elastin and Masson's trichrome staining was carried out to evaluate the presence of elastin and collagen respectively in decellularized tissue. Furthermore, MTT test was done to assess the in vitro scaffold's cytocompatibility.
Results: According to the results of H&E staining, the bovine ovarian cortex was decellularized in all groups. No residual nuclei were observed by DAPI staining. Preservation of the ECM was evaluated by Masson Trichrome and Gomori's aldehyde-fuchsin staining. Elastic and collagen fibers were kept after the decellularization process in all groups. OD values of eluted formazan of MTT test showed that fibroblasts on the SDS-Triton-Amonium decellularized scaffolds were more viable than other groups. Human ovarian cortex was decellularized completely with mentioned protocol. Furthermore elastic and collagen fibers were kept in decellularized human ovarian cortex too. There was no significant difference in the Proliferation rate of the fibroblast cells in the human decellularized scaffold and two-dimensional conventional culture system.
Conclusion: In conclusion, human ovarian cortex was decellularized with the combination of different SDS solution and the bovine ovarian cortex was decellularized by SDS, SDS-Triton and SDS-Triton-Ammonium. Structure of ECM was not damaged in any groups. However, MTT test results of SDS-Triton-Ammonium were better than other groups.
Key words: *Fertility preservation, Ovarian cortex.*

O-23
Comparison of pre and postnatal rat model of polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is considered to be a gynecological condition and is the commonest endocrinopathy in reproductive-aged women, with an estimated prevalence ranging between 5-15% worldwide. Well-developed animal models that closely mimic human diseases e.g., PCOS may provide useful data for this approach; such investigations are impossible in humans. The aim of this study was to determine one of the most suitable rat models of PCOS that closely mimics human PCOS phenotype, in order to further investigations about PCOS.
Objective: The aim of this study was to determine one of the most suitable rat models of PCOS that closely mimics human PCOS phenotype, in order to further investigations about PCOS.
Materials and Methods: We searched Pubmed, Science direct, and Web of science between 1990 and 2016, for relevant English manuscripts, using keywords including the "Polycystic Ovary Syndrome AND Rat Model" to generate a subset of citations relevant to our research. Included were those articles that compared at least both ovarian histology or estrous cycle and reproductive hormonal profiles in hormone-induced rat model of PCOS and controls.
Results: Differences in the findings between hormone-induced PCOS rats appear to be a result of the degree of transplacental transfer of the steroid administered into the fetus, dose and type of hormone, route of administration and timing and duration of exposure.
Conclusion: We conclude that prenatal hormone-induced rat model with a lower dose and shorter time of exposure during the critical period of fetal development that exhibits endocrine, ovarian and metabolic disturbances similar to PCOS in women, while maintaining normal reproductive system morphology in adulthood is more suitable than postnatal hormone-induced rat model to facilitate studies regarding PCOS.
Key words: *Hormone, PCOS, Rat, Review.*

O-24
Morphological, ultrastructural, and molecular aspects of in vitro mouse embryo implantation on human endometrial mesenchymal stromal cells in the presence of steroid hormones as an implantation model

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Background: Implantation is a complex process that involves fine coordination and dialogue between the embryo and endometrium. Embryonic development to the blastocyst stage and uterine differentiation to the receptive phase are both essential for initiation and progression of a successful implantation. The process of implantation consists of apposition, adhesion, and the invasion of the blastocyst to the uterine wall.
Objective: This experimental study aimed to evaluate the effects of 17a-estradiol (E2) and progesterone (P4) on the interaction between mouse embryo and human endometrial mesenchymal stromal cells, and gene expressions related to implantation [alpha-V and beta-3 integrins, interleukin-1 receptor (IL-1R), and leukemia inhibitory factor receptor (LIFR)] using an in vitro two dimensional model.
Materials and Methods: In this experimental study, the endometrial stromal cells were isolated enzymatically and mechanically, and cultured to the fourth passage. Next, their immunophenotype was confirmed by flow cytometric analysis as mesenchymal stromal cells. The cells were cultured as either the experimental group in the presence of E2 (0.3 nmol) and P4 (63.5 nmol) or control group without any hormone treatment. Mouse blastocysts were co-cultured with endometrial mesenchymal stromal cells in both groups for 48 hours. Their interaction was assessed under an inverted microscope and scanning electron microscopy (SEM). Expressions of alpha-V and beta-3 integrins, LIFR, and IL-1R genes were analyzed by real-time reverse transcription-polymerase chain reaction (RT-PCR).
Results: Similar observations were seen in both groups by light microscopy and SEM. We observed the presence of pinopode-like structures and cell secretions on the apical surfaces of endometrial mesenchymal stromal cells in both groups. The trophoblastic cells expanded and interacted with the mesenchymal monolayer cells. At the molecular level, expression of IL-1R significantly increased in the hormonal treated group compared to the control ($p \leq 0.05$). Expressions of the other genes did not differ.
Conclusion: This study has shown that co-culture of endometrial mesenchymal stromal cells with mouse embryo in media that contained E2 (0.3 nmol) and P4 (63.5 nmol) could effectively increase the expression of IL-1R, which is involved in embryo implantation. However, there were no significant effects on expressions of alpha-V and beta-3 integrins, LIFR, and on the morphology and ultrastructure of endometrial mesenchymal stromal cells.
Key words: *Estrogen, Implantation, Interleukin-1 receptor, Mesenchymal stromal cells, Progesterone.*

O-25
Effect of catheter rotation during its withdrawal on frozen thawed embryo transfer cycles outcomes; case-control study

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Background: Embryo transfer is the final and most clinical process in assisted reproductive technology (ART) cycle. Cervical mucus has been suggested to interfere with adequate embryo transfer in different ways. A few studies implied that catheter rotation could discharge mucus entrapped in the embryo so as to thwart embryo displacement.
Objective: In this retrospective study we compared the outcome of frozen embryo transfer (FET) base on catheter rotation during withdrawal.

Materials and Methods: In the present case-control retrospective study, the clinical documents of 240 subjects (women) who experienced FET cycles. The subjects were divided into two groups, including (A) the rotation treatment group (n=120, 360 degrees) that underwent ET using catheter rotation, and (B), the control (n=120), including the subjects who experienced ET with no catheter rotation. In the end, clinical and chemical pregnancies and implantation rates were compared between the two groups.
Results: The basic clinical and demographic features of both groups had no differences of significance. A significant difference was observed between both groups in terms of the rate of chemical pregnancy (21.7% vs. 43.3%, p=0.001) and implantation rate (18.47% vs. 8.19%, p=0.002). In addition, in the study group, the rate of clinical pregnancy was significantly higher (33.3% vs. 14.2%, p=0.002).
Conclusion: The results of data analysis in this study demonstrated that the rotation of the catheter when withdrawn increased the rates of implantation and clinical pregnancy.
Key words: *Embryo transfer, Assisted reproductive technic, Implantation, Pregnancy.*

O-26

Comparison of Notch1-3, genes expression and Jagged-1,2 proteins in cumulus cells of PCOS and healthy women's and their correlations with oocyte, fertilization and embryo

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Background: Bi-directional communication between the oocyte and cumulus cells is essential for the production of competent oocytes and corresponding embryos. In this regard, apoptosis of cumulus cells has a negative effect on oocyte and embryo quality. One of the signaling pathways that plays important roles in maintaining of differentiated cells and reducing apoptosis is the Notch pathway, but so far, little is known about this signaling pathway in cumulus cells and its correlation with the oocyte and embryo in patients with polycystic ovary syndrome.
Objective: To investigate associations between gene expression pattern of Notch signaling receptors and its ligands in cumulus cells of polycystic ovary syndrome (PCOS) patients and the quality of oocytes, zygotes and embryos.
Materials and Methods: 40 intracytoplasmic sperm injection (ICSI) patients, of whom 20 were PCOS and 20 were healthy women, included in this study. Serum hormone levels were measured by using Radioimmunoassay (RIA) for each patient. The expression of NOTCH1, NOTCH2 and, NOTCH3 in 200 cumulus complexes surrounding mature oocytes was examined by real-time polymerase chain reaction (real-time PCR). Further, immunohistochemistry was performed for Jagged-1 and Jagged-2 proteins.
Results: The expression levels of Notch pathway genes and proteins including NOTCH 2,3, and Jagged-1, Jagged-2 proteins were significantly different between groups) $p<0.05$. (There was a statistically positive significant correlation between the level of NOTCH-2 and zygote quality (r=0.802, p=0.042). In addition, there were positive correlations between NOTCH1-3 and embryo quality (r=0.445, p=0.005, r=0.311, p=0.058, r=0.332, p=0.042). There were no correlations between Jagged-1 and Jagged-2 expressions and oocyte quality ($p>0.05$) while Jagged-2 showed a negative correlation with zygotes with Z3 grade (r=-0.128, p=0.044). In addition, Jagged-2 showed a positive correlation with top embryo quality (r=0.199, p=0.023) and negative correlation with embryo with grade2(r=-0.320, p=0.051).
Conclusion: This study reveals that the measurement of Notch pathway genes and proteins levels in cumulus cells could be used as genetic biomarkers for oocyte and embryo selection under an ART program.
Key words: *PCOS, Oocyte quality, Embryo quality, Zygote quality, Cumulus cells.*

O-27

The effect of pharmacological and combined psychological approach in the treatment of unconsummated marriage secondary to vaginismus

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Background: Among female sexual dysfunction disorders, unconsummated marriage (UCM) secondary to primary vaginismus is a common sexual dysfunction especially in traditional societies.
Objective: According to various clinical evidences, patients with UCM usually do not respond to conventional treatment methods and need to some further psychological interventions.
Materials and Methods: Forty one women, aged 18 to 40 years with a diagnosis of UCM secondary to primary vaginismus (genito-pelvic pain/penetration disorder) who failed in previous treatments including hymenectomy surgery, sedative drugs, lubricants and anesthetic creams enrolled in this study through

convenient available sampling method. In this research we investigated a combination of psychological approach including behavior therapy techniques (relaxation training, systematic desensitization and exposure), cognitive intervention and hypnotherapy with prescription of psychotropic medication. The treatment period consists of 8-16 sessions (2-4 months) which held once a week. Data collection were done through interview and statistical analysis was done through using descriptive statistics and Fisher exact test.
Results: Success rate in this approach was 82.9%. Three of these patients with a history of UCM for 10, 12 and 16 years have had successful treatment and two of them become pregnant after 10 and 12 years. Based on the results, age, education, traditional or non-traditional marriage and duration of illness have no statistically significant relationship with success rate but at the same time lack of other psychiatric disorders including anxiety, depression and obsessive-compulsive disorder was significantly associated with more successful treatment (P<0.05).
Conclusion: Application of combined psychological approach with pharmacotherapy seems to be more effective in the treatment of UCM patients.
Key words: *Unconsummated marriage, Primary vaginismus.*

O-28
Evaluation of Toll-like receptor 3 (TLR3) signaling pathway genes and its genetic polymorphisms in ectopic and eutopic endometrium of women with endometriosis

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Background: It has been proven that innate and adaptive immune responses dysregulation has an undeniable role in endometriosis. Toll-like receptors (TLRs, as members of the innate immune system) are expressed in the human endometrium and their aberrant regulation and expression are involved in the pathogenesis of endometrial diseases.
Objective: This study aimed at evaluation of TLR3 signaling pathway genes and its genetic changes in women with endometriosis.
Materials and Methods: The case-control study included 176 patients: 83 cases with endometriosis and 93 healthy fertile women. Blood samples were collected from all subjects and polymerase chain reaction (PCR) was performed in blood-derived DNA for detection of single nucleotide polymorphisms (SNP) of TLR3. The PCR products were sequenced by Macrogen Company (South Korea). Also, endometriotic lesions from the ectopic ovarian endometrioma (EC) and eutopic endometrial (EU) biopsies from the same subjects (n=20) were obtained from endometriosis women, as well as endometrium from healthy women (n=16, as control group, CE) in the proliferative phase of menstrual cycle (days 5-14). Quantitative PCR was performed for determination of mRNA expression level of TLR3 signaling pathway genes (TLR3, TICAM, NF-kB1A, CXCL10, IRF3, IFN-B1, IL-6 and IL-8 genes) in tissue samples. Also, serum protein levels of TLR3, IFN-β, IL-6 and IL-8 were determined using Enzyme Linked Immunosorbent Assay (ELISA).
Results: The mRNA gene expression levels of TLR3, NF-kB1A, IFN-B1, IRF3, TICAM1, IL-6 and IL-8 were significantly higher in Eutopic endometrium compared to ectopic ones and also compared to CE ($p<0.05$ or $p<0.01$). There was no significant difference in CXCL10 expression between EU vs. CE whereas its mRNA expression was significantly higher in EU than ectopic ones. We examined the 1.7 kb length of TLR3 gene. The results showed two SNPs including one (rs3775291) which causes amino acid substitutions Leu >Phe and a synonymous (Phe=) SNP (rs3775290). Results from PCR and gene sequencing showed that SNPs frequency was not significantly different between patients and controls. Serum protein levels of TLR3, IFN-β, IL-6 and IL-8 were significantly increased in patients with endometriosis in comparison to the control group ($p<0.05$).
Conclusion: In this study, moreover to elevation of IL-6 and IL-8 cytokines, significant and clear changes was observed in the mRNA expression of other genes in TLR3 cascade in diseased eutopic endometrium, demonstrating that EU similarly to EC was in an intensive inflammatory state. Interestingly, the results showed that expression of aforementioned genes have significant difference between ectopic and eutopic endometrium of endometriosis patients, suggesting that fundamental alterations in the concept of immune response in eutopic endometrium may lead to its activation and escapes from apoptosis. These changes maybe have potential contribution to misplaced implantation of endometrium, and then ectopic tissues become stable to a certain degree from the immunological point of view. Also, the results showed that two SNPs (rs3775291 and rs3775290) in TLR3 gene have not impact on its mRNA expression in endometriotic endometrium.
Key words: *Endometriosis, Toll like receptor 3, Polymorphism, Signaling pathway.*

O-29
Correlation between sperm motility and sperm chromatin/DNA damage before and after cryopreservation and the effect of folic acid and nicotinic acid on post-thaw sperm quality in normozoospermic men

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Background: Cryopreservation exposes sperm to physical and chemical stresses due to the overexposure of reactive oxygen species and reduced efficacy of existing antioxidants.
Objective: We aimed in this experiment to study the association between sperm motility and sperm chromatin/DNA damage before and after cryopreservation. Then, we investigated the possible protective effects of nicotinic acid and folic acid on sperm quality.
Materials and Methods: Semen samples were collected randomly from 30 normozospermic males with age range of 25-45 yr. Each sample was divided into 5 groups: fresh, cryopreserved without treatment (control), with nicotinic acid or folic acid, and combination of folic acid and nicotinic acid. In each group to assess sperm viability and motility we used eosin-nigrosin staining and computer aided sperm analysis (CASA), and for chromatin and DNA quality we used aniline blue and toluidine blue staining, sperm chromatin dispersion test and acridine orange staining.
Results: Sperm quality after cryopreservation showed statistically significantly reduced in comparison to fresh sample groups ($p<0.05$). Before and after cryopreservation, sperm chromatin/DNA damage was negatively correlated with percentage of progressively motile cells. After addition of folic acid or nicotinic acid in cryopreservation medium found to significantly improved sperm parameters and DNA and chromatin quality when compared with the control groups ($p<0.05$), the combination of folic acid and nicotinic acid showed best cryoprotective effect on semen.
Conclusion: In conclusion, significant reduction in progressive motility in post-thaw sperm can be indicator of significant chromatin/DNA damage and folic acid and nicotinic acid showed to have cryoprotective effect on sperm quality.
Key words: *Cryopreservation, Sperm, DNA, Chromatin, Folic Acid, Nicotinic Acid.*

O-30
The effects of hydrocortisone on tight junction genes in an in vitro model of the human fallopian epithelial cells

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Background: The tight junction between epithelial cells helps making connections in the fallopian tube and contributes to successful fertilization. Breaking the tight junction complex induces various diseases such as the EP. Previous studies have shown that glucocorticoids are effective in repairing and maintaining intercellular tight junctions in epithelial cells of the fallopian tube, although their mechanism is still unknown.
Objective: This research is a genomic study of hydrocortisone's effect on epithelial cells of the fallopian tube.
Materials and Methods: Using the human fallopian tube, epithelial cell line (OE-E6/E7) was cultured in four concentrations of hydrocortisone (0 nM, 50 nM, 100 nM and 200 nM) for three durations (24, 48 and 72 hr).
Results: Glucocorticoids are effective on the expression of Zona occluding-1 (ZO-1), Claudin 4, Claudin3, Desmoglein and E-cadherin genes involved in the tight junctions of the fallopian tube. The expression of all genes was up-regulated in the concentrations of 100nM after 48h treatment, as compared with the control (0nM). However, their expression was down-regulated significantly after 72h treatment ($p<0.05$).
Conclusion: The present study showed that treatment of epithelial cells of the fallopian tube with glucocorticoid increased the expression of genes involved in tight junctions, including claudin-3, claudin-4, E-cadherin, zona occludin-1 and Desmoglein-1. The obtained data suggests that a new mechanism is developed for glucocorticoid induction of tight junctions by increasing the expression of claudin-3, claudin-4, E-cadherin, zona occludin-1 and Desmoglein-1 genes.
Key words: *Hydrocortisone, Tight Junction gens, Fallopian Tube, Epithelial Cell, Gene Expression.*

3rd Congress of Reproductive Genetics

O-31
Frequency of AZF microdeletions in azoospermic men candidate for micro TESE

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Background: The Y chromosome accumulates male-related genes including sex-determining region of Y-chromosome (SRY) and several spermatogenesis-related genes. The long arm contains azoospermia factor (AZF) region (including sub-regions AZFa, AZFb and AZFc).
Objective: Microdeletions in this region are responsible for azoospermia and oligospermia and results in the male infertility. The aim of this study was to analyze incidence of microdeletions in the AZF region of Y chromosome in patients with azoospermia from Iran.
Materials and Methods: Over the period from 2016 to 2018 a total of 358 men were analyzed. The diagnosis of azoospermia was established on the basis of semen

analysis. All patient samples were analyzed cytogenetically. Chromosomal analysis was performed on all patients on cultured lymphocytes from peripheral blood. For exact diagnosis of microdeletions in AZF region we used a PCR-method using a set of sequence-tagged sites from all AZF sub-regions (according to the recommendation by the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN).

Results: Among of all patients with azoospermia and normal karyotype, 11% of patients had microdeletions in the AZF region of the Y chromosome. Among of all patients with microdeletions were 3.9% microdeletions in the AZFc region, 2.5% microdeletions in the AZFb region and 1.2% microdeletions in the AZFa region. Men with isolated AZFc deletion, sperm were found in 50% by mTESE, although in some cases they did not have a good morphology.

Conclusion: The study confirmed that percentage of microdeletions in the AZF region in Iranian azoospermic patients is similar to the global rate and Microdeletion of the entire AZFa or AZFb regions of the Y chromosome portends an exceptionally poor prognosis for sperm retrieval, whereas the majority of men with AZFc deletion have sperm within the semen or testes available for use in IVF/ICSI.

Key words: *AZF, mTESE.*

O-32
Overexpression of STAT3 gene in women with endometriosis

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Background: Endometriosis is an inflammatory disease that characterized as the growth of the endometrial tissue outside the uterine cavity. It has shown that inflammation plays important role in pathogenesis of endometriosis. Several studies revealed that different cytokines and chemokines which involved in inflammation and angiogenesis are increased in endometriosis. One of these cytokines is interleukin- 6 (IL-6) which is increased in endometrial tissue and peritoneal fluid of women with endometriosis. Another one is IL-17 which is shown its increase in peritoneal fluid of patients with endometriosis. Signal transducers and activators of transcription3 (STAT3) is a transcription factor which is activated by some cytokines signaling pathways such as IL-6 signaling. On the other hand, STAT3 as a transcription factor influences on expression of some genes such as IL-17.

Objective: The objective of this study was to investigate the gene expression of STAT3 in endometrial tissues of women with endometriosis compared to control ones.

Materials and Methods: In this case control study, 20 women with endometriosis and 20 women without endometriosis were enrolled after laparoscopy as endometriosis and control groups, respectively. Ectopic endometrial samples were obtained through laparoscopic procedure while eutopic and control endometrial tissues were obtained by pipelle. The mRNA expression of STAT3 in control, eutopic and ectopic endometrial samples were quantitatively evaluated by real time polymerase chain reaction (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.P value less than 0.05 was considered as statistically significant.

Results: The findings showed that mRNA expression of STAT3 was statistically increased in ectopic tissues of endometriosis women compared to control and eutopic endometrial samples ($p<0.05$). In addition, mRNA expression of STAT3 was increased in eutopic endometrial tissues of endometriosis group compared to control endometrial samples but this difference was not statistically significant ($p>0.05$).

Conclusion: Regarding to the results of this study, it seems that overexpression of STAT3 gene in endometriotic tissues of women with endometriosis may be involved in pathogenesis of endometriosis. For getting more information, we need to study this gene in a large number of women with and without endometriosis and also to investigate gene expression of other factors involved in signaling pathways of inflammatory cytokines. Epigenetic studies such as STAT3 binding to the promoter regions of target genes such as IL17 are recommended.

Key words: *Endometriosis, STAT3, Ectopic, Eutopic, Endometrium.*

O-33
The effect of medium on the rate of chromosomal instability in human amniotic fluid cells

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Background: Recent developments in regenerative medicine have led to the search for stable, safe and highly accessible stem cell sources with therapeutic potential. Although the genetic stability of stem cells to be used in cell therapy and other clinical applications is strongly critical, but the relationship between in vitro expansion of

stem cell lines and their genomic stability has not yet been completely elucidated.

Objective: The present paper aims to validate the possible role of medium on the rate of chromosomal instability in human amniocytes until development of replicative senescence.

Materials and Methods: Amniotic fluid samples were donated by genetics laboratories of Yazd reproductive sciences institute. We received the samples at first passage when amniocentesis procedure had been over. Each sample was cultured with two different medium: 1) DMEM supplemented by 4 mM L-Glu, 10 mM HEPES, 15% FBS, 1% PenStrp; 2) AmnioMAX II supplemented by 10 mM HEPES and 1% PenStrp. The samples were harvested between passages 4-9 when they had been senescent and not be able to be passaged anymore. Afterwards, the cells were fixed on slide and evaluated considering eight probes for X-13/18/21 and Y-15/16/22 chromosomes by the FISH technique.

Results: Our results showed that total chromosomal abnormality around 9.9% and 4.3% in DMEM and AMX respectively. Investigating 535 and 510 cells in samples from two medium showed overall 75 abnormal cells more with polyploidy.

Conclusion: Overall, according to potential of hAFCs and derived- stem cells and regarding to low frequency of chromosomal abnormalities and high stability of them in long term cultures, we would suggest applying them in cell therapy and regenerative medicine research.

Key words *Amniotic fluid cells, Chromosomal instability, FISH, Amniomax, DMEM.*

O-34
The relationship between the expression levels of miR-135a and HOXA10 gene in the eutopic and ectopic endometrium

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Background: The study of microRNA expression can be effective in the diagnosing and treating different diseases. miR-135a is one of the most important micro-ribonucleic acids involved in endometriosis. Among the genes that become the target of the miR-135a and are subjected to changes in the endometrium of patients with endometriosis is HOXA10 gene which is expressed in the endometrium in response to steroid hormones.

Objective: The aim of this study was to evaluate the expression of miR-135a and its relationship with the level of HOXA10 gene expression in both endometrial ectopic and eutopic tissues in patients with endometriosis compared to the control samples.

Materials and Methods: In this prospective case-control study, both case-eutopic and case-ectopic tissue samples were obtained from 17 women with endometriosis and the eutopic endometrial tissue was sampled from 17 women with normal endometrium as the control group. The gene's expression of miR-135a and HOXA10 were investigated using quantitative reverse transcription PCR (q-RT PCR).

Results: A significant decrease in the expression of HOXA10 gene was detected in case-eutopic during the luteal phase compared to the control samples (p=0.001), while in the case-ectopic, the expression of this gene was increased (p=0.681) compared to the control samples. In addition, the expression miR-135a in the luteal phase showed a remarkable increase in the case-eutopic endometrial tissue (p=0.026) as well as a significant decrease in the case-ectopic endometrial tissue compared to the control samples (p=0.008).

Conclusion: Considering the inverse relations between the over-expression of miR-135a and the reduction of HOXA10, it seems that miR-135a may be applied as an endometrial diagnostic and therapeutic biomarker.

Key words: *Endometriosis, Gene expression, Micro-ribonucleic acid, HOXA10, miR-135a.*

O-35
Supplementation of sperm freezing medium with Myoinositol improve human sperm parameters and protects it against DNA fragmentation and apoptosis

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Background: Cryopreservation has been extensively used in assisted reproductive technology and it is an important task to improve current methods of sperm cryopreservation. Myoinositol is involved in several

systemic processes and in mechanisms of signal transduction in the plasma membrane as precursor of second messengers. On the male reproductive function, MYO appears to regulate seminal plasma osmolarity and also sperm maturation, motility, capacitation and acrosome reaction. Recently an antioxidant action has also been suggested.

Objective: The aim of this study is to evaluate the beneficial effect of MYO supplement in freezing media on the post thaw sperm quality.

Materials and Methods: Semen samples from 40 normozoospermic men were divided into two aliquots and frozen with 2mg/ml MYO free /or supplemented freezing medium. Post thaw process, computer-aided semen analysis was used to analyze sperm motility and morphology. Reactive oxygen species (fluorometry of DCFH-DA), total antioxidant capacity, lipid peroxidation (colorimetric assay by ELISA reader) and DNA fragmentation (TUNEL staining) were evaluated.

Results: MYO significantly improved progressive motility and normal morphology in treated samples ($p<0.05$). Lipid peroxidation can be precluded in samples frozen with MYO supplemented freezing media ($p<0.05$). While MYO did not affect significantly the amount of ROS ($p>0.05$), it was associated with a significant increase in total antioxidant capacity ($p<0.05$). In MYO treated samples, DNA fragmentation was lower than control ones ($p<0.001$).

Conclusion: The findings support the use of 2mg/ml myoinositol supplemented freezing media in sperm cryopreservation to increase sperm quality after freezing-thawing procedures.

Key words: *Myoinositol, Human sperm cryopreservation, Sperm parameters, Oxidative stress, DNA fragmentation.*

O-36
Aneuploidy screening of arrested embryos derived from in-vitro matured oocytes

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Background: High rates of aneuploidy have been reported either as adverse effects of in vitro manipulation or part of physiological in vivo development. However, there was no information on the rate of aneuploidy in IVM derived embryos that is one of the reasons for discarding immature oocytes in ICSI cycles. On the other hand, also, the association between embryo morphology and aneuploidy remains uncertain.

Objective: This study aimed at evaluating aneuploidy and quality of arrested embryos derived from in-vivo and rescue in-vitro matured oocytes.

Materials and Methods: In this study, 30 and 35 arrested cleavage stage embryos in case and control group were respectively analyzed by FISH technique for detecting aneuploidies in X, Y, 13, 15, 16, 18, 21 and 22 chromosomes.

Results: There were insignificant differences between aneuploidy rates of embryos in both groups ($p>0.05$), regarding to sex, autosome and total abnormalities also from the point of view chaotic and mosaicism. Moreover, chromosomal abnormality was observed in a high percentage of good quality embryos from both groups, without correlation between the morphological characteristics of embryos and chromosomal content.

Conclusion: Our finding demonstrated neither embryo quality nor aneuploidy rate differ significantly between in vivo and in vitro derived embryo. Therefore, we would suggest IVM as a valuable and practical option for patients who have had to cancel IVF treatment cycles because of severe responses or resistance to routine hormonal therapies or those with low functional ovarian reserve.

Key words: *IVM, Embryo Quality, Aneuploidy, FISH.*

O-37
A novel mutation found in a patient with congenital adrenal hyperplasia

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Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders demonstrated by a failure in one of the five enzymes responsible for cortisol production. The disorder represents itself by low blood levels of estrogens, androgens, and cortisol that generally pair with hypertension, Hypokalemia, sexual infantilism and primary amenorrhea in affected individuals.

Objective: A 14-yr-old female, the first child of consanguineous parents with normal family history was referred to genetic clinic with high blood pressure, ambiguous genitalia, and lack of pubertal development. No pubic or axillary hair was seen by physical examination, and she had no clinical symptoms of Turner syndrome. She was hypertensive (150/90 mmHg, 50th percentile for age) with high gonadotropins levels (LH, 19 mU/mL; FSH, 34 mU/mL). Moreover, low peripheral concentrations of sex steroids were seen.

Materials and Methods: Sonographic and karyotyping method was performed. Genomic DNA was isolated from peripheral blood. All eight exons of CYP17A1 gene were multiplied by PCR. Products were sequenced.

Results: The karyotype was normal (46, XX) and in sonographic survey uterus was infantile. Sanger Sequence chromatogram of the CYP17A1 gene show A

new in-frame homozygous deletion c.1052-1054CCT in exon 6 (deletion of 351Leu in protein and CCT deletion on cDNA sequence).
Conclusion: Deficiency of CYP17A1, the hormonal alterations are summarized as sex steroids and cortisol insufficiencies with mineralocorticoids excess. This case manifested typical feature of 17α-hydroxylase and 17,20-lyase deficiencies. This study report an in-frame deletion which leads to isolated 17,20-lyase deficiency and this mutation may be used for diagnosis in other patients with typical clinical symptoms. Diagnosis of 17α hydroxylase/17,20 lyase deficiency was confirmed by the specific profile of adrenal steroid levels, and further confirmation by CYP17A mutation analysis.
Key words: *Congenital adrenal hyperplasia (CAH), CYP17A1 gene, Ambiguous genitalia.*

O-38
Fluorescence in-situ hybridization detects increased sperm aneuploidy in men with recurrent pregnancy loss in the Iranian population

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Background: The definitions of recurrent pregnancy loss (RPL) are traditionally defined as three or more consecutive pregnancy losses. Despite numerous studies, RPL etiology remains obscure and the presence of an etiologic factor seldom suggests a conclusive diagnosis of the cause. While all of proposed etiological factors have been extensively explored, the male contribution to recurrent abortion at the sperm chromosome level has rarely been examined.
Objective: Our purpose was to assess the existence of sperm autosome and sex chromosome aneuploidy in recurrent pregnancy loss (RPL).
Materials and Methods: In this prospective study, 15 men with recurrent pregnancy loss and 5 men with normal sperm analysis, without abortion history and with at least one child were included. Two- and three-color fluorescence in situ hybridization (FISH) technique was used for screening aneuploidy in 13, 18, 21, X, and Y chromosomes.
Results: In total, the percentage of sperm aneuploidy was significantly increased in men with RPL than normal men. Among analysed chromosome, prevalence of nollisomy of chromosome 13 was higher rather other studied chromosomes. As well, our results showed that normal karyotype in blood cells does not exclude the presence of sperm chromosomal abnormalities.
Conclusion: These results suggest an implication of sperm chromosome abnormalities in some cases of recurrent pregnancy loss. The increased incidence of sperm chromosome abnormalities observed in the recurrent abortion patients does not rule out other factors such as maternal contribution. However, according our results, we would like to suggest evaluating sperm aneuploidy in couples with unexplained recurrent abortions especially with the ones with chromosomally abnormal aborted fetus.
Key words: *RPL, Male factor, Aneuploidy of sperm, FISH.*

O-39
Increased expression of BCL6 gene in endometrial tissues of women with endometriosis

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Background: Endometriosis is one of the most common diseases of women in reproductive age which characterized as growth of endometrial glands and stroma outside of the uterine cavity. Some symptoms of endometriosis are pelvic pain, dysmenorrhea, dyspareunia, dysuria and infertility. Different cellular alterations are reported in endometriosis including increased inflammation, cell proliferation, decreased apoptosis and changes in immune cells. B-cell lymphoma 6 (BCL6) is a transcriptional factor that inhibits apoptosis and promote cell proliferation and differentiation. On the other hand, interleukin 6 (IL-6) is one of the inflammatory cytokines and some studies have shown that IL6 level is increased in peritoneal fluid of women with endometriosis. Binding of IL-6 to its receptor recruited signal transducer and activator of transcription 3 (STAT3) and it has been reported that STAT3 upregulates BCL6.
Objective: Collectively, the objective of this study was to evaluate BCL6 gene expression in eutopic and ectopic endometrium of women with endometriosis compared with the endometrium of control group.
Materials and Methods: In this study, 10 women with endometriosis and 10 women without the disease (control group) who underwent diagnostic laparoscopy at Royan Institute were included. Ectopic endometrial samples were collected during laparoscopy while eutopic and control endometrial samples were collected by pipelle. RNA extraction and cDNA synthesis were done for all tissue samples then gene expression of BCL6 was studied using real time PCR. Data analysis was done using one way ANOVA. $P<0.05$ was considered statistically significant.
Results: The expression level of BCL6 gene was increased in ectopic and eutopic endometrial tissues of

women with endometriosis compared with control samples ($p<0.05$ and $p>0.05$ respectively). In addition, BCL6 gene expression was higher in ectopic tissues than eutopic ones in endometriosis group.

Conclusion: These results revealed that over-expression of BCL6 gene in endometrial tissues of women with endometriosis could contribute to the pathogenesis of endometriosis.

Key words: *Endometriosis, BCL6, Ectopic, Eutopic, Endometrium.*

O-40
High incidence of sperm aneuploidy revealed by array CGH in oligoasthenoterato-zoospermic (OAT) patients

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Background: Aneuploidy is a main cause of miscarriage and implantation failure whilst contribution of sperm aneuploidy in sterility is not well defined. High incidence of aneuploidy has been reported in OAT spermatozoa using fluorescence in situ hybridization (FISH). OAT is associated with lower pregnancy and implantation rates. Limited number of chromosomes is an inherent disadvantage of FISH studies.

Objective: All chromosomes have not been studied in spermatozoa from OAT patients simultaneously. We investigated comprehensive aneuploidy screening of spermatozoa in OAT by array comparative genomic hybridization (aCGH).

Materials and Methods: Four severe OAT patients without Y chromosome microdeletions with normal blood karyotype and history of implantation failure were enrolled. The female partners were healthy aged <36. Illumina 24 sure platform aCGH was employed for three samples of each patient containing 2 spermatozoa. Depending on the aneuploidies found by aCGH, FISH was utilized to find the magnitude of the detected abnormalities in total population of spermatozoa.

Results: Aneuoploidy was detected by aCGH in 82% of samples ranged 1-6 chromosomes per patient. The most frequent ones were chromosomes 8, 21(3 of 4 patients; 36.4% and 45.5% of samples, respectively). We observed abnormalities that hardly ever considered in FISH studies of sperm such as chromosomes 19 and 20. Range of 782-1286 sperm per patient were scored in FISH, the most frequent abnormalities were disomy of chromosomes 13, 20, 22 and X/Y with mean rates of 10.9%, 6.2%, 5.2% and 5.6%, respectively and total aneuploidy was 47.55-81.6%.

Conclusion: Both aCGH and FISH revealed incident aneuploidy in OAT spermatozoa. Aneuploidies of the single sperm seem to be independent of frequent aneuploidies in total sperm population. Regarding the high incidence of aneuploidies in severe OAT, comprehensive preimplantation aneuploidy screening should be more investigated in OAT.

Key words: *Sperm, Aneuploidy, Array CGH, FISH, Oligoasthenoteratozoospermia*

O-41
The relationship between mutation in *CPS1* gene and pregnancy loss

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Background: Carbamoyl phosphate synthetase 1 (CPS1) is a liver-specific enzyme with the lowest enzymatic rate, which determines the overall rate of the other reactions in the pathway that converts ammonia to carbamoyl phosphate in the first step of the urea cycle. Carbamoyl-phosphate synthetase 1 deficiency (CPS1D) which usually presents as lethal hyperammonemia, is a rare autosomal recessive hereditary disease.

Objective: Here we report a case of a two-day-old female neonate with lethal hyperammonemia. The newborn infant presented with hyperammonemia. In Plasma amino acid analysis, there was a significant elevated level of alanine, aspargine, glutamic acid, aspartic acid and lysine. We cannot diagnose the urea cycle disorder (UCD) carbamoyl-phosphate synthetase 1 deficiency (CPS1D) properly only based on the quantity of biochemical intermediary metabolites to exclude other UCDs with similar symptoms.

Materials and Methods: We performed Single Nucleotide Polymorphism (SNP) array and after that we performed a whole exome genome sequencing based on next generation sequencing. Sanger sequencing to confirm the mutation was in the patient (homozygous). The mutation was checked in her parent and other family members too.

Results: We found a novel missense c. 2758G>C mutation in exon 23 of CPS1 at amino acid position 920 (p. Asp920His). At the end, we used the Sanger sequencing to confirm the mutation was in the patient (homozygous). The mutation was checked in her parent and other family members too.

Conclusion: We applied whole exome sequencing (WES) successfully to diagnose the patient with CPS1D in a clinical setting. This result supports the clinical applicability of WES for cost-effective molecular diagnosis of UCDs in prenatal diagnosis to future procedures of disease-free embryo selection.

Key words: *Carbamoyl-phosphate synthetase 1 deficiency, CPS1, Urea cycle disorder, Next generation sequencing, Biochemical test.*