Award Winners *(Alphabetic order)*

A-1

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

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

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

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

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

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

A-2

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

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

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

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

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

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

A-3

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

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

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

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

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

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

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

A-4

The immunomodulatory effects of decidual microenvironment on dendritic cells

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

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

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

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

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

A-5

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

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

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

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

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

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

A-6

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

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

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

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

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

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

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

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

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

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

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

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

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

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Introduction: Endometriosis, the growth of endometrial-like cells outside the uterus, is thought to occur due to differential regulation of gene expression in ectopically growing tissues. Although endometriosis is a multifactorial disease and the exact etiology is not clearly understood, recently, some evidence suggests that epigenetic is associated with the molecular features of endometriosis.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

A-11

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

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

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

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

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

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

A-12

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

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

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

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

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

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

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

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

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

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