Poster Presentations *(Alphabetic order)*

8th Yazd International Congress and Student Award in Reproductive Medicine

1- Andrology

P-1

Assessment of genetic variations of exons 8-13 of *SUN5* gene and its protein expression in men with acephalic spermatozoa syndrome referring to Royan institute

Aalinia S[1], Hosseini S[2], Sadighi Gilani MA[2], Sabbaghian M[2].
1.*Department of Basic Science and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*
2.*Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: marjan.sabbaghian@gmail.com

Background: Infertility is a major problem in worldwide that affected 10-15% of couples. Approximately 40% of infertility issues are due to male factors. Therefore, evaluation of the causes of male infertility is important. One of the main causes of male infertility is associated with abnormal sperm morphology. Teratozoospermia is a condition characterized by the presence of spermatozoa with abnormal morphology in semen samples. Acephalic spermatozoa is a rare and severe form of teratozoospermia, that identified by headless tails and spermatozoa with an abnormal head-tail junction. The genetic cause of this disorder in human was unknown. Recent studies indicate that mutations in *SUN5* (Sad1 and UNC84 domain containing 5) gene probably lead to acephalic spermatozoa syndrome. *SUN5* gene encode a testis-specific protein and appears to play a role in the meiotic stage of spermatogenesis. It has essential role in junction between sperm head and tail. Also probably involved in nuclear envelope reconstitution and nuclear migration. Thus, this gene is an appropriate candidate in human studies.
Objective: The purpose of current study was to evaluate the genetic variations of *SUN5* gene. Thus, exons 8, 9, 10, 11, 12 and 13 of *SUN5* gene were investigated in 20 infertile men with acephalic spermatozoa as case group and 20 fertile men as control group.
Materials and Methods: Accordingly, we extracted DNA from blood samples, designed primers and performed PCR reactions, after that sanger sequencing was done and results were analyzed by Finch TV. Furthermore, we compared the protein expression in patients and control group by using western blotting.
Results: Sequence results identified one homozygous variant in exon 13 (c.1073G>A [p.Arg358Gln]). This variant causes amino acid changing and leads to missense mutation. We didn't identify mutations in control group and the other case group. To confirm the results, evaluating protein expression by western blotting was done. We observed complete deletion of protein in mutant sample compared with normal protein.
Conclusion: According to our findings and previous studies, it could be concluded that the mutations of SUN5 gene are led to acephalic spermatozoa syndrome, but these mutations are not existing in all patients, therefore it recommends to evaluate of other genes that could be led to acephalic spermatozoa syndrome.
Key words: *Male infertility, Teratozoospermia, Acephalic spermatozoa, SUN5 gene.*

P-2

Myoinositol can improve freeze sperm parameters in patients with Oligoasthenoteratospermia

Abdolsamadi M.
Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: mona.abdolsamadi2@gmail.com

Background: Myoinositol is a sugar-like compound from family of vitamin B complex. Myoinositol is mainly produced via Sertoli cells in response to Follicle-stimulating hormone. This antioxidant play an important role in regulation of motility, total antioxidant capacity, and DNA fragmentation in sperm cells. The purpose of this study is evaluation of the effect of Myoinositol on Cryopreservation of sperm in patients with Oligoasthenoteratospermia syndrome.
Objective: Effect of Myoinsitol on freeze sperm parameters in patients with Oligoasthenoteratospermia.
Materials and Methods: Semen samples were obtained from 40 patients with oligoasthenoteratospermi (OAT) syndrome and each sample was divided into two groups after macroscopic and microscopic analysis. in the first group, semen sample combined with only freezing medium (control group) and in the second group, semen sample mixed with freezing medium and 2 mg/ml Myoinositol (experimental group). semen samples after thawing were investigated for semen parameters with CASA analysis, reactive oxygen specifics (ROS) by DCFH-DA fluorometry, Total antioxidant capacity (TAC) and MDA test for lipid peroxidation by ELISA and DNA fragmentation with TUNEL assay.
Results: After the thawing process, the Total and progressive Motility of sperm significantly increased in the myoinositol group compared with the control group. Myoinositol could not affect ROS and MDA in a significant manner, but it could increase TAC significantly. TUNEL results showed that DNA integrity was significantly maintained by Myoinositol. DNA fragmentation was decreased in myoinositol group (22.44) compared to the control group (29.67) ($p<0.001$).
Conclusion: It seems that myoinositol have an effect on sperm motility, TAC and prevent increasing DNA fragmentation in freezing process. So, it could be a good component in sperm freezing process in oligoastenoteratospermic patients.
Key words: *Oligoasthenoteratospermi syndrome, Myoinositol, Cryopreservation.*

P-3

Cryoprotective effect of sericin supplementation in freezing and thawing media on the outcome of cryopreservation in human sperm

Aghaz F, Khazaei M, Vaisiraygani A, Bakhtiari M.
Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: miana512000@yahoo.com

Background: The destructive effects of sperm cryopreservation results in decreased sperm parameters and their fertilizing ability. Antioxidants supplementation can potentially improve cryopreservation outcomes.
Objective: In this study, we tried to investigate the effects of sericin supplementation in freezing and thawing media on frozen-thawed human sperm motility, morphology, viability and DNA fragmentation.
Materials and Methods: In experiment 1, semen samples were collected from 30 healthy fertile men and were cryopreserved in the presence of freezing medium supplemented with different concentrations of sericin (0, 0.5, 1, 2.5 and 5%).
Results: The results showed that the addition of 2.5 and 5% sericin in freezing medium significantly increased sperm viability and total motility (A+B) and decreased DNA fragmentation ($p<0.05$). In experiment 2, semen samples were collected from 21 fertile men and were cryopreserved in freezing medium without any supplementation for 48h. Then, the samples were thawed in medium supplemented with different concentrations of sericin (0, 0.5, 1, 2.5 and 5%). The addition of 5% sericin to thawing medium increased the total motility, viability, and decreased DNA fragmentation compared with those in thaws without sericin.
Conclusion: In nutshell, the results clearly indicate the feasibility of sericin as cryoprotective supplement for freezing media in human spermatozoa.
Key words: *Sericin, Sperm cryopreservation, DNA fragmentation, Oxygen reactive species, Antioxidant.*

P-4

Study of Nrf2 antioxidant gene in testicular tissue of Wistar male rats induced by varicocele

Ahmadzadeh Golsefied A, Eskandari A, Nasr-Esfahani MH, Tavalaee M.
Royan Institute for Biotechnology, Isfahan, Iran.
Email: mh.nasr-esfahani@royaninstitute.org

Background: According to the World Health Organization (WHO) Infertility is defined as the inability to achieve a pregnancy after one year of unprotected sexual intercourse. IN the world almost 4% of men suffer from infertility. Varicocele is an abnormal inflation of the pampiniform venous in the scrotum that is considered as one of the main causes of infertility in men, and are associated with increased levels of oxidative stress that can lead to the expression of antioxidant genes in these individuals.
Objective: The purpose of this investigation was to create a varicocele model in animals and to study the levels of oxidative stress and Nrf2 antioxidant gene expression in those.
Materials and Methods: In the present study, 30 adult male Wistar rats were divided into three groups, I: varicocele-induced II: sham and III: control. After two months of varicocele induction, rats were sacrificed and epididymis were dissected. Then, sperm lipid peroxidation assessed by bodipy test. In addition, Real Time PCR technique was used to determine the expression of the n gene of the RNA level. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Tukey). Collected data were presented as mean±SEM and $p<0.05$ was considered to be significant.
Results: The results of the current study showed that level of lipid peroxidation in sperm higher in varicocele induction group compared to control and sham groups ($p<0.01$). Expression of Nrf2 antioxidant gene was significantly higher in varicocele induction group compared to control and shame groups ($p<0.01$).
Conclusion: In the varicocele status due to the high testicular temperature, and consequently increase of oxidative stress level, antioxidant genes such as Nrf2 was activated to counteract the increased oxidative stress and lipid peroxidation in sperms. So Nrf2 is an important to spermatogenesis and may use in the diagnosis of male's infertility.
Key words: *Infertility, Varicocele, Lipid peroxidation, Nrf2 gene.*

P-5

Protective effect of combined pumpkin seed and ginger extracts on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with cyclophosphamide

Amini Mahabadi J[1], Aghaie S[2], Nikzad H[1].
1. *Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
2. *Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
Email: hosseinnikzad43@yahoo.com

Background: Reproductive toxicity is one of the side effects of cyclophosphamide (CP) in cancer treatment. Pumpkin seeds and Zingiber officinale are natural sources of antioxidants.
Objective: We investigated the possible protective effect of combined pumpkin seed and Zingiber officinale extracts on sperm characteristics, epididymal histology and biochemical parameters of CP-treated rats.
Materials and Methods: Male adult Wistar rats were divided randomly into six groups. Group 1, as a control, received an isotonic saline solution injection intraperitoneally (IP). Group 2 were injected IP with a single dose of CP (100 mg/kg) once. Groups 3 and 4 received CP plus 300 and 600 mg/kg combined pumpkin seed and Zingiber officinale extract (50:50). Groups 5 and 6 received only 300 and 600 mg/kg combined pumpkin seed and Zingiber officinale extract. Six weeks

after treatment, sperm characteristics, histopathological changes and biochemical parameters were assessed.
Results: In CP-treated rats, motile spermatozoa were decreased, and abnormal or dead spermatozoa increased significantly ($p<0.001$) but administration of the mixed extract improved sperm parameters. Epididymal epithelium and fibromascular thickness were also improved in extract-treated rats compared to control or CP groups. Biochemical analysis showed that the administration of combined extracts could increase the total antioxidant capacity (TAC) level significantly in groups 3, 4, 5 and 6. Interestingly, the mixed extract could decrease most of the side effects of CP such as vacuolization and separation of epididymal tissue.
Conclusion: Our findings indicated that the combined extracts might be used as a protective agent against CP-induced reproductive toxicity.
Key words: *Cyclophosphamide, Pumpkin seed and Zingiber officinale extract, Rat's epididymis, Sperm parameters, Total antioxidant capacity.*

P-6
Evaluation of ART devices toxicity with human sperm motility assay

Anbari F, Tofighi M, Izadi M, Mahaldashtian M, Maleki B, Khalili MA.
Department of Reproductive Biology, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: Khalili59@hotmail.com

Background: To maintain the highest standard in the ART laboratory, all consumables utilized for culture of embryos and gametes should be examined in terms of quality control. In this study, human sperm motility assay (HSA) was used for evaluating toxicity of consumable devices.
Objective: Evaluation of falcon 15ml, Petri dish 10cm, Surgical gloves, tip, filtered tip, Iranian Petri dish, Ultrasonography probe and sperm sampling container toxicity with Human sperm motility assay.
Materials and Methods: Eight consumable devices in the ART Laboratory were investigated by HSA test. These devices were included falcon 15ml, Petri dish 10cm, Surgical gloves, tip, filtered tip, Iranian Petri dish, Ultrasonography probe and sperm sampling container. In this study, we used normal sample of sperm with more than 50% mobility and after swim up, the specimens were exposed to the device for 10 min. Both control and case groups were examined after 10 min, 30 min, 1, 2, 4 and 24 hr. In each group, the percentage of motile sperm was evaluated. Then the ratio of sperm motility in case to control group was considered as an index of toxicity. If the ratio was less than 85%, the intended device was considered to be toxic. This test was performed triplicate.
Results: The results of our study showed that after the above-mentioned times, there were no significant difference between the case and control groups in falcon 15 ml, Petri dish 10 cm, tip, filtered tip and sperm sampling container. Both surgical gloves and Iranian petri dish after 24 hr and ultrasonography probe after 2 and 24 hr, were detected toxic.
Conclusion: Considering the importance and sensitivity of the ART laboratory, Qualitative control tests must be carried out on consumable device. In this regard, HSA is one of the tests to be considered.
Key words: *Toxicity, Human sperm motility assay, HSA, Surgical gloves, ART devise.*

P-7
The efficiency of daily intake of selenium, vitamin E and folic acid on the sperm parameters: A blinded clinical trial study

Bahmyari R, Kavianie M, Azima S.
Department of Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: azimas@sums.ac.ir

Background: Since 1940, many pieces of literature have introduced reactive oxygen species as the pivotal factors in the pathophysiology of 30-40% of males with idiopathic infertility. In order to confront with excessive generation of ROS, various antioxidants have been examined to suppress detrimental production of ROS and improve sperm quality and the chance of fertilization.
Objective: Evaluation the effect of daily intake of selenium, folic acid and vitamin E on the sperm parameters.
Materials and Methods: This randomized, blinded, placebo-clinical trial study was conducted by 70 men who were diagnosed as idiopathic infertile patients and conferred to clinics of urology affiliated to Shiraz University of Medical Sciences and met the inclusion criteria. They were conducted to daily intake of 200µgr, 400 I.U and 5mgr folic acid for three months. After three months semen analysis was repeated by SQAV and data was analyzed.
Results: We assessed the effect of daily intake of 200 µgr of selenium, 400 units of vitamin E and 5 mg of folic acid on the sperm parameters of infertile men once at the beginning of the study and once three months after the incidence of treatment. We detected no significant improvement in the sperm concentration ($p=0.12$), total motility ($p=0.171$), progressive motility ($p=0.767$), SQAV morph ($p=0.48$), SMI ($p=0.375$) and functional sperm ($p=0.087$) between the experimental and the control group at the end of experiment. Significant improvement in semen volume ($p=0.038$) and microscopic morph ($p=0.003$) was observed. In order to detect the real enhancement in the semen volume and microscopic morph, difference in difference test was applied. The results showed no significant amendment in semen volume ($p=0.61$) and sperm microscopic morph ($p=0.90$) between the experimental and control group at the end of the intervention.
Conclusion: Based on our results, the treatment has not been effective in improving the sperm parameters in patients with oligoasthenoteratospermia. Further investigations with larger sample size is required to assess the efficacy of these antioxidants on the sperm parameters more accurately.
Key words: *Male infertility, Antioxidants.*

P-8
The effects of FSH, LH, Testosterone on DFI in oligospermia

Bahrami Z.
Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: znbahrami@gmail.com

Background: Male factors are significantly related to 50% of the cases attending infertility Clinics. High levels of DNA fragmentation are often related to declined fertilization rates and embryo development. Evidence suggests that human sperm chromatin anomalies adversely affect reproductive outcomes. Hormones have pivotal roles for spermatogenesis regulation. Spermatogenesis is initiated by the endocrine influence of gonadotrophins that act on the Sertoli cells of the testis.
Objective: The aim of this study is the evaluation of the relationship between male reproductive hormones including FSH, LH, and Testosterone with sperm parameters, DFI and consequently its application in infertility treatment.
Materials and Methods: Semen samples were taken from men undergoing infertility evaluation for male factor (n=30) and non-male factor (n=30) infertility. 60 male infertility cases of Laleh Hospital in two groups had oligo-spermia, and the control group had normal sperm parameters. These samples were evaluated for sperm parameters using a light microscope and used in TUNEL assay to detect DNA fragmentation in sperm. Blood samples were taken for hormonal assay by ELISA method. Serum samples were analyzed for FSH, LH and Testosterone.
Results: Finally, correlation between hormones including FSH, LH, and Testosterone and sperm parameters in addition to DFI was analyzed. Sperm DNA damage is moderately increased in male factor infertility. Our results showed direct relation between the level of FSH, LH and DFI. Excessive increased or decreased testosterone levels were associated with increased DNA damage.
Conclusion: Therefore, according to the results of this study, hormonal changes including FSH, LH and Testosterone can reflect DFI and quality of sperm. As a final result, in infertile couples, assessment of hormones and DFI should be done in advance to any clinical approaches.
Key words: *DNA Fragmentation Index (DFI), Sperm parameters, Infertility, Hormones.*

P-9
Effect of co- administration of pentoxifylline and zinc on sperm parameters and DNA fragmentation in idiopathic male infertility

Dadgar Z[1], Soleimani Mehranjani M[1], Shariatzadeh M[1], Rahsepar M[2], Ghazali Sh[2], Kheirolahi A[4].
1.*Department of Biology, Faculty of Science, Arak University, Arak, Iran.*
2.*ART Laboratory, Center of Infertility and Recurrent Abortion, Shafa Hospital, Khorramabad, Iran.*
3.*Department of Urology, Lorestan University of Medical Sciences, Khorramabad, Iran.*
Email: z.dadgar67@gmail.com

Background: Pentoxifylline is one of methyl xanthine derivative which influences the sperm motility and commonly used in treatment of male infertility with asthenozoospermia. Zinc as a known antioxidant is also a DNA condenser which influences spermatogenesis. it is believed that consumption of Pentoxifylline can affect integrity of sperm DNA.
Objective: We aimed to evaluate the possible effect of a combination of zinc and Pentoxifylline on sperm parameters and DNA integrity in a group of patients with asthenozoospermia.
Materials and Methods: Ninety men with asthenozoospermia in a doubleblind, randomized clinical trial were allocated for this study. They were randomly divided into three groups including: Pentoxifylline, Pentoxifylline +zinc and zinc. Orally (twice daily) treatment carried out for a period of 3 months. Finally, we compared the sperm parameters and DNA fragmentation using sperm chromatin dispersion (SCD) test before and after treatment. Data was analyzed statistically using one-way ANOVA and Tukey's test and means difference was consider significantly different at $p<0.05$.
Results: Pentoxifylline and Zinc increased significantly the mean sperm motility, count and normal morphology in the Pentoxifylline and zinc treated groups when compared to pre-administration. The mentioned parameters increased significantly in the Pentoxifylline + Zinc group in comparison with Pentoxifylline and Zinc groups. A significant increase and decrease in sperm DNA fragmentation was found in the Pentoxifylline and zinc treated groups compared to pre-administration respectively. There was no significant difference in the mean sperm DNA fragmentation in Pentoxifylline + Zinc group before and after administration.
Conclusion: Our results showed that Pentoxifylline although has improved the sperm motility but it may have some adverse effect on sperm DNA quality. However, the co-administration of pentoxifylline and zinc could protect sperm DNA from undesirable effects of pentoxifylline on sperm DNA quality. Therefore, consumption of Pentoxifylline with antioxidants such as zinc is suggested in the case of male infertility treatment.
Key words: *Pentoxifylline, Zinc, Asthenozoospermia, DNA Fragmentation.*

P-10
Association between body mass index and sperm miR-34c transcript content

Davoodi E[1], Dorostghoal M[1], Galehdari H[2], Hamadi M[3].
1.*Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, Iran.*
2.*Department of Genetic, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*
3.*Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
Email: elahe.davoodiii69@gmail.com

Background: Obesity has been considered as an important factor contributing male infertility. Present study was done to investigate the relationship between body mass index (BMI) and sperm miR-34c transcript content in normozoospermic men.
Objective: Present study was done to investigate the relationship between body mass index (BMI) and sperm miR-34c transcript content in normozoospermic men.
Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. BMI were measured in a total of 30 men attending an infertility clinic and categorized to two groups with normal weight (18.5-24.9 kg/m^2) and overweight (25.00-29.9 kg/m^2). Standard sperm parameters were analyzed according to the World Health Organization (WHO) guidelines and expression levels of miR-34c were assessed in ejaculated sperm.
Results: Expression of miR-34c was significantly ($p<0.05$) higher in men with normal BMI than overweight men. The sperm content of miR-34c showed significant ($p=0.020$, $r=0.422$) relationship with BMI.
Conclusion: Our findings show the association between sperm content of miR-34c and body mass index and suggest that obesity could affect sperm microRNAs expression profile.
Key words: *Body mass index, Obesity, Male fertility, Semen quality, miR-34c.*

P-11

Is there any relationship between human sperm parameters and protamine deficiency in different groups of infertile men?

Dehghanpour F[1], Fesahat F[2], Yazdinejad F[1], Motamedzadeh L[1], Talebi AR[1].
1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: prof_talebi@hotmail.com

Background: Abnormality in Histone- Protamine replacements have been indicated to cause sperm DNA damage and infertility.
Objective: The aim of present study was to investigate the relationships between sperm parameters in oligospermia, asthenospermia, and teratospermia with protamine deficiency in infertile men.
Materials and Methods: In this case-control study, we had three experimental groups including oligospermia (n=100), asthenospermia (n=100), and teratospermia (n=100) and also normospermia (n=100) as controls. Sperm analyses were done according to the recommendations of the World Health Organization (WHO, 2010) and sperm chromatin quality was assessed using Chromomycin A3 (CMA3) staining for each sample.
Results: Sperm concentration and normal morphology were significantly different between all experimental groups as well as controls ($p<0.05$), except those between oligospermia and asthenospermia semen samples. The comparison of the data between groups indicated that the percentage of spermatozoa with protamine deficiency was significantly different in patients with oligospermia, asthenospermia, and teratospermia when compared with control ones. However, There was no significant difference between sperm nuclear protamine deficiency of the men with teratospermia and oligospermia using CMA3 test ($p=0.172$).
Conclusion: The higher proportion of spermatozoa with abnormal chromatin packaging was observed in asthenospermic samples than those from other experimental as well as control. The data indicate that chromatin quality assessments could apply as the key confirmatory indicator beside the semen analysis in infertile men, as well.
Key words: *Sperm, Protamine deficiency, Teratozoospermia, Oligospermia, Asthenospermia.*

P-12

The impacts of cell phone radiation on human sperm parameters, chromatin quality, and apoptosis in normozoospermic samples

Doostabadi M[1], Hassanzadeh-taheri M[2], Khalili MA[1], Hosseininejad Mohebati A[3], Zardast M[4].
1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Anatomy, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.*
3.*Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran.*
4.*Department of Pathology, Faculty of Medicine Birjand University of Medical Sciences, Birjand, Iran.*
Email: doostabadim@yahoo.com

Background: Nowadays, there is an increase in infertility in human societies, and this pattern is related to several factors. One of the environmental factors suggesting for male infertility is frequent contact with radiofrequency magnetic radiation (RF-EMR) that emitted from some devices like cell phone. These waves have potential adverse effects on some body's organs such as brain, heart and testes.
Objective: This study aimed to assess the effects of mobile waves on human sperm parameters, quality of chromatin and apoptosis in normal semen samples.
Materials and Methods: In this cross-sectional study 60 normal sperm samples were divided into two equal parts as the case and control. Samples of intervention group were placed at distance of 2.5 cm close to a cell phone for 60 minutes in active antenna mode. There was no intervention for the control group. Then, all specimens from both groups were analyzed by spermogram and percentages of abnormal sperms and sperm motility were recorded. Moreover, sperm viability, sperms with additional histone and stable chromatin as well as sperm apoptosis were assessed.
Results: Sperm viability and motility rates in the intervention group were significantly lower than the control group ($p<0.001$, $p=0.004$ respectively) while the mean percentage of apoptotic sperms in the intervention

group was significantly higher than the control group (p=0.031). The other studied parameters showed no significant difference between the two groups.
Conclusion: RF-EMR of mobile phone can reduce the sperm's viability and its motility and also induce apoptosis. Therefore, based on the harmful effects, it is suggested that the cell phone be kept away from the body.
Key words: *Cell phone, Sperm motility, Apoptosis, Semen.*

P-13
Sperm PTEN transcript content in normozoospermic fertile and infertile men

Dorostghoal M, Danyari R.
Department of Biology, Faculty of Sciences, Shahid Chamran University, Ahvaz, Iran.
Email: Dorostghoal@gmail.com

Background: It is believed that routine sperm analysis has limited power to predict reproductive health. So, assays to detect genetic abnormalities have been developed for providing molecular data to clarify pathogenesis of unexplained infertility.
Objective: Present study evaluated sperm PTEN content in normozoospermic fertile and infertile men.
Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. Semen samples were collected from fertile (n=15) and infertile men (n=15) with normal sperm parameters according to the World Health Organization (WHO) guidelines. Real-time PCR was carried out to evaluate relative expression of PTEN mRNA in ejaculated sperm.
Results: Sperm motility was significantly ($p<0.05$) lower in men with unexplained infertility compared with control group. Content of PTEN transcript in ejaculated sperm was significantly ($p<0.05$) higher in infertile men than fertile control. Also, significant negative correlation ($r=-0.408$, $p=0.025$) was seen between PTEN transcript content and sperm motility.
Conclusion: This study suggests that assessment of PTEN sperm content transcript has significant predictive value as a complementary diagnostic tool in evaluation of fertility in normozoospermic infertile men.
Key words: *Unexplained male infertility, Normozoospermia, Sperm Transcriptome, PTEN.*

P-14
Human semen hyper viscosity and sperm motility

Eshaghi Sh[1], Mohammadi P[1, 2, 3], Bagheri N[1], Torkiyan N[1], Naghshineh E[1, 4].
1. *Maryam Infertility Center, Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
3. *Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
4. *Department of Obstetrics and Gynecology, Infertility Center, Beheshti University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: shahlaeshaghi133@gmail.com

Background: The prevalence of semen hyper viscosity (SHV) is estimated to be between 12-29% and can lead to male factor infertility both in vivo and in vitro. Infection and high levels of seminal leukocytes may results in the development of SHV. Oxidative stress and biochemical and genetic factors can furthermore contribute to this condition.
Objective: The aims of this study were (a) to determine the prevalence of subjects with semen hyper viscosity (SHV) in a large population of male partners of subfertile couples; (b) to identify any correlation between SHV and infections or inflammation of the genital tract and (c) to assess sperm kinetic parameters of SHV.
Materials and Methods: Semen samples were collected and examined after liquefaction for 20 min at 37°C from patients in Andrology Laboratory of Maryam Infertility Center, Beheshti Hospital, Isfahan, Iran. One hundred fifty-eight Semen samples of male partners of subfertile couples with increased seminal viscosity and 129 Semen samples with normal seminal viscosity as controls.
Results: Sperm count (38.6 vs. 20.1) and motility (45.5 vs. 31.0) were significantly lower in patients with hyper viscosity compared to the control group. There was positive correlation between SHV and infections or inflammation of the genital tract.
Conclusion: OS and high seminal ROS levels in SHV are related to SHV decreased sperm parameters such as motility and increased sperm DNA damage. Hyper viscosity can impair normal sperm movement in the female reproductive tract, and can lead to decreased sperm count. SHV is most attributed to male accessory gland infection, increased levels of leukocytes, and inflammation, as well as dysfunction of the sex glands or even the immune system.
Key words: *Semen hyper viscosity, Progressive motility, Male infertility, Semen parameters.*

P-15
Evaluation of genetic variations in exons 1-7 of SUN5 gene and its protein expression in men with acephalic spermatozoa referring to Royan Institute

Eskandari Chenari F[1], Sadighi Gilani MA[2], Javid A[1], Sabbaghian M[2].
1. *Department of Biology, Science and Arts University, Yazd, Iran.*
2. *Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: marjan.sabbaghian@gmail.com

Background: Acephalic spermatozoa syndrome is a one of the most severe forms of teratozoospermia which cause male infertility; it can be easily defined through detection of decapitated flagella, tailless sperm heads in the ejaculated. In this syndrome, the sperm's head is separated from the flagellum because there is a problem in head-tail junction. This condition was caused by a defect in the spermatogenesis stage in the testicle. It is an autosomal recessive type defect and probably has genetic

implications in many cases. Furthermore, SUN5 (Sad1 and UNC84domain containing 5) produces a testis-specific protein that localized in the mature spermatozoa head-to-tail linkage site and plays a role in the attachment of the head-tail of the flagellum during spermatogenesis.
Objective: In this study we investigated the variations of exons 1,2,3,4,5,6,7 of SUN5 gene and their related protein expression in infertile men with acephalic spermatozoa syndrome.
Materials and Methods: In our study 20 infertile men with acephalic sperm syndrome and 30 normal fertile men as control groups were recruited. In order to do this study, we extracted DNA from peripheral blood using salting out method, designed primers and performed PCR reactions, after that Sanger-sequencing technique was done and results were analyzed by Finch TV. Furthermore, we also performed quantitative protein expression of SUN5 using Immunocytochemistry analysis for case and control groups.
Results: Results of sanger-sequencing revealed no mutations or single nucleotide polymorphism (SNPs) between men with acephalic spermatozoa and control individual. We observed the expression of SUN5 protein at the linkage between head-tail, because we have not seen any mutation in these arise of the SUN5 gene, so we expected that no expression changes of protein have observed between case and control groups.
Conclusion: Although the present study demonstrated that there is no relationship between the genetic variation of exons 1-7 SUN5 gene and acephalic spermatozoa syndrome, since SUN5 is necessary for head- tail junction, it seems for a closer look it should be suggested to examine other exons in this gene, introns, splice sites and the promoter.
Key words: *SUN5 gene, Male infertility, Acephalic spermatozoa syndrome.*

P-16
The effect of L-carnitine on sperm DNA structure, chromatin quality and apoptosis in BALB/C mice treated with formalin

Ezati Yazdani D, Vardiyan R, Talebi AR, Anvari M.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: daniyalezatiyazdani1369@gmail.com

Background: Formalin is widely used as tissue fixative and disinfectant. It contains reactive molecules which have been known for its cytotoxic effects, According to recent studies, formalin causes a change in the testicular and sperm structure; with the effects of L-carnitine as an antioxidant.
Objective: Our aim was to evaluate the effect of L-carnitine on DNA structure, Chromatin quality and apoptosis in BALB/C mice treated with formalin.
Materials and Methods: In this interventional study, 32 adult BALB/C mice (10-12 wk old; 30-35gr weight) were randomly divided into 4 groups of 8 mice each. The control group received no medicine. Sham group; received only serum normal saline 10 ml/kg intraperitoneally for 31 days. The 3rd group was exposed to formalin, 10 mg/kg injected intraperitoneally for 31 days. The 4th group were exposed to formalin, 10 mg/kg intraperitoneal injection daily and L-carnitine (100 mg/kg solution) for up to 31 days. Then the mice were killed by spinal cord injury and sperm samples were taken and then sperm chromatin condensation and DNA integrity were assessed by four different tests including chromomycin A3 (CMA3), toluidine blue (TB), aniline blue (AB) and TUNEL for sperm apoptosis.
Results: The results showed that formalin increase the percentage of sperm cells with abnormal chromatin ($p \leq 0.05$). In addition, in comparison with control group, we had a significant elevation in spermatozoa with residual histones (assessed by AB). With the aid of staining tests, we found that with using formalin in mice, there was a significant increase in DNA damage in comparison to the control group. In addition, with regard TUNEL test, the rate of reacted spermatozoa was increased significantly in L-carnitine group when compared with the controls ($p<0.05$).
Conclusion: Formalin disturbs nuclear maturity and DNA integrity of spermatozoa. Therefore, the production of spermatozoa with less condensed chromatin and more apoptotic rate increases after formalin exposure and this may be one possible cause of infertility following formalin exposure, and L-carnitine improve this so it can be used as a prophylaxis in these individuals.
Key words: *Formalin, L-carnitine, DNA structure, Chromatin, apoptosis.*

P-17
Evaluation of sperm parameters, chromatin quality and apoptosis rate in men with a history of morphine abuse

Ghasemi-Esmailabad S[1], Gholizadeh L[2], Moshrefi M[2], Amiri S[1], Tabibnejad N[2], Talebi AR[2].
1. *Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproduction Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: prof_talebi@ssu.ac.ir

Background: Consumption of opioid drugs is considered as a significant factor affecting the male fertility. Morphine, as an essential alkaloid of opium structure has adverse effects on the human sperm and semen parameters. Furthermore, oxidative stress generated by opioids may affect sperm DNA integrity and chromatin quality.
Objective: To evaluate the possible effects of morphine abuse on sperm parameters, chromatin quality and DNA integrity in opium addicted men.
Materials and Methods: The study included 30 opiate addict men and 30 healthy volunteers as controls. The case subjects were categorized into three different groups: men who abused opium (group 1); men with a history of addiction to both opium and cigarette (group 2); and men

addicted to both opium and its derivatives (opium extract) with a history of cigarette smoking (group 3). Semen collection and analysis were performed according to World Health Organization criteria (2010). Routine semen parameters such as sperm count and motility were evaluated by Makler chamber. To determine the sperm chromatin abnormalities, aniline blue (AB) and toluidine blue (TB) staining methods were applied. The percentage of apoptotic spermatozoa was evaluated by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Then data were statistically analyzed by one-way analysis of variance (ANOVA), Student's t test as well as Mann-Whitney U test. $p<0.05$ was considered significant.

Results: Sperm count and total motility were significantly decreased in the case groups compared with controls ($p<0.05$). However, no significant differences were found between the subgroups of the case subjects regarding sperm count and total motility ($p>0.05$). There was a significant increase in the rate of aniline blue-positive spermatozoa as well as toluidine blue-positive spermatozoa in the opium addicts compared to the controls ($p<0.05$). Moreover, case group had a higher percentage of spermatozoa with apoptosis (TUNEL positive) compared to the controls significantly ($p<0.05$). However, there were no significant differences between case subgroups with regard to aniline blue and toluidine blue staining in addition to TUNEL assay ($p>0.05$).

Conclusion: The results indicated that morphine abuse may have deleterious effects on semen parameters and can adversely affect the sperm chromatin and DNA integrity regardless of the kind of opiates.

Key words: *Opium addiction, Morphine, Sperm chromatin, Oxidative stress, Apoptosis.*

P-18
MSOME detected fine sperm abnormalities in infertile men with high BMI

Ghazali Sh[1], Khalili MA[2], Talebi AR[3], Anbari F[2], Nabi A[2], Halvaei I[4], Mangoli E[2], Ghasemzadeh J[3].

1.*Department of Midwifery, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran.*
2.*Department of Reproductive Biology; Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: sh_ghazali@yahoo.com

Background: Studies have shown that body mass index (BMI) may have a negative correlation with sperm parameters. It becomes possible to assess spermatozoa morphology at high magnification (×6600) with motile sperm organelle morphology examination (MSOME), Using this technique, a comprehensive information can be recorded about spermatozoa anatomy, especially head region.

Objective: The aim was to investigate the relationship between BMI and various sperm parameters according to MSOME criteria in infertile men.

Materials and Methods: A total of 76 men presented as male factor with average age of 34.4±6.1. They were categorized in 3 group of men I: normal weight (BMI: 18.5-24.9 kg/m^2), II: overweight (BMI: 25-29.9 kg/m^2), III: obese (BMI: ≥30 kg/m^2). MSOME criteria were compared between 3 groups as the presence and size of vacuoles (none, small, medium, large, mix), head size (normal, small, large), Presence or absence of cytoplasmic droplets; head shape (normal or abnormal) and acrosome status. Sperm parameters were assessed according WHO criteria. Protamine deficiency was also assessed in sperm chromatin by chromomycin A3 (CMA3) staining.

Results: There were significant differences between groups II and III for large head size ($p=0.028$) but rate of cytoplasmic droplets, head shape, acrosome status and sperm parameters were no difference in 3 groups. Also, the data showed a significant correlation between BMI with large vacuoles in spermatozoa ($r=0.23$, $p=0.043$) and large head size ($r=0.24$, $p=0.036$). High level of BMI had a negative correlation with sperm motility, but it was insignificant ($r=-0.039$, $p=0.74$). Sperm chromatin status in 3 groups were similar ($p=0.56$).

Conclusion: Obesity is associated with both large vacuoles and large head size in spermatozoa in infertile men. MSOME, is a useful tool for accurate selection of spermatozoa for ICSI program.

Key words: *Body mass index, Male infertility, Obesity, MSOME criteria.*

P-19
Effects of vitamin D supplementation on semen quality and reproductive hormones: A randomized double-blind placebo-controlled clinical trial

Gheflati A, Nadjarzadeh A, Mirjalili S, Riahi H, Hosseini E, Salehi-Abargouei A.

Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: azadehnajarzadeh@gmail.com

Background: Animal models and observational studies have suggested a favorable role of vitamin D and male reproduction. However, randomized clinical trials regarding the effect of vitamin D supplementation on male fertility are rare.

Objective: The aim of this study was to examine the effect of vitamin D supplementation on semen quality, reproductive hormones and anthropometric measurements in infertile men with deficient vitamin D levels.

Materials and Methods: 44 individual males with infertility were randomly assigned to treatment group (supplemented with 9 pearls of vitamin D containing 50000 IU vitamin D3) or control group (received 9 pearls of placebo), one pearl per week for 12 weeks. Semen quality markers (sperm count, morphology, sperm motility, sperm volume), total testosterone, sexual hormone binding globulin (SHBG), free androgen index (FAI), and anthropometric measurements (weight, Body mass index (BMI), waist circumference (WC), Hip

circumference (HC), waist to hip ratio (WHR) were measured at baseline and end of the study.
Results: Serum 25 (OH) D levels of were significantly higher in men in the treatment group compared with the placebo group. In a multivariate adjusted model, vitamin D supplementation decreased WC significantly in comparison to control group. However, no statistical significant differences were seen in changes of semen quality markers (sperm count, morphology, sperm motility, sperm volume), total testosterone, sexual hormone binding globulin (SHBG), FAI and other anthropometric values ($p>0.05$).
Conclusion: Vitamin D supplementation did not improve semen quality markers, reproductive hormones and other anthropometric measurements in vitamin D-deficient infertile men compared to control group. Larger and longer randomized clinical trials (RCTs) are needed to investigate the possible effects of vitamin D supplementation in treatment of infertility.
Key words: *Vitamin D, Male infertility, Semen quality, Reproductive hormones.*

P-20
Relationship between micro-particle contaminations with semen quality and assisted reproductive outcomes

Gholizadeh L, Agha-Rahimi A, Sabour M, Khani F, Khalili MA.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: khalili59@hotmail.com

Background: Bacterial contaminations of human semen are quite frequent and may contribute to the deterioration of the semen quality. The presence of bacteria in semen may also interfere with infertility treatment and influence the development and viability of human embryos and pregnancy outcomes. However, there have been conflicting reports regarding the effects of bacterial contaminations on sperm fertility potential.
Objective: To determine the possible relationship between seminal bacterial contaminations with conventional sperm parameters, fertilization and cleavage rate, embryo quality and subsequent pregnancy in infertile men.
Materials and Methods: A total of 120 infertile men attending for assisted reproductive cycles were included in this study. Out of these, 50 men with the bacteriospermia and 70 men with no apparent clinical signs of contamination were considered as the case and control groups, respectively. The presence of micro-particle contamination in semen samples were assessed according to previous reports with phase-contrast light microscopy. Small sphere-shaped particles were observed in the head, midpiece and tail of the spermatozoa, which were completely different from the debris routinely visible in semen specimens. These micro-particles were interpreted as bacterial contamination. Sperm count and motility were evaluated by Makler chamber. Diff-Quik staining method was applied for sperm morphology. After completion of semen and bacteriologic analysis, the retrieved oocytes were fertilized by ICSI or IVF procedures. The fertilization and cleavage, embryo quality and pregnancy rates were recorded. Data were analyzed using Student's t test and Mann-Whitney U statistical methods.
Results: Normal morphology and progressive motility of spermatozoa were significantly decreased in the case group compared with controls ($p<0.05$). Case group had higher percentage of immotile spermatozoa in comparison with controls significantly ($p<0.05$). However, no significant differences were found between the case and control subjects regarding other sperm parameters ($p>0.05$). In a similar trend, there were no significant differences between the case and control groups regarding the fertilization, cleavage, embryo quality, and pregnancy rates ($p>0.05$).
Conclusion: Presence of the micro-particles in semen was associated with compromised semen quality in terms of sperm motility and morphology. However, these particles did not interfere with clinical outcomes.
Key words: *Semen quality, Bacteriospermia, Assisted reproduction, ICSI.*

P-21
The effect of Inofolic on intracellular reactive oxygen species (ROS) and reduced glutathione (GSH) levels in the MII oocyte of DHEA-induced PCOs mice

Haghighi M[1, 2], Mehdizadeh M[1], Amjadi FS[1], Zandieh Z[1], Mohammadi F[1].
1.*Department of Anatomy, Iran University of Medical Sciences, Tehran, Iran.*
2.*Jahad Daneshgahi lorestan,Khorramabad, Lorestan,Iran.*
Email: nbsmmsbn@iums.ac.ir

Background: Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism, polycystic ovaries and ultimately oocytes whit poor quality. The administration of myo-inositol was associated with a decreased of serum testosterone, increase insulin sensitivity and improves the oocytes' quality.
Objective: The purpose of this study is to determine the effects of inofolic on oocyte ROS and GLUT levels in PCOs model.
Materials and Methods: The present study was performed in DHEA-induced mice. Female NMRI mice were treated with a vehicle control (Sesame Oil 0.05 ml and saline) or DHEA (6 mg /100 g body weight) or DHEA plus inofolic (37 mg /100g body weight) for 20 consecutive days. After 20 days superovulation was performed, Mature oocysts (MII) were retrieved from isolated ovaries for determination of intracellular reactive oxygen species (ROS) and reduced glutathione (GSH) levels.
Results: Respectively GSH and ROS were significantly lower and higher in DHEA-treated oocytes compared with vehicle-treated. In DHEA+inofolic group, ROS was significantly reduced in contrast to the DHEA group and, On the contrary, GLUT has risen.

Conclusion: It seems that the inofolic plays an important role in reducing ROS and increasing the antioxidant capacity of mice oocytes in the PCOS model.

Key words: *Inofolic, Oocyte, Reactive oxygen species, Reduced glutathione, PCOS mice model.*

P-22

The effect of non-essential amino acid supplementation on the parameters and DNA fragmentation index of cryopreserved sperm

Hamidi D.
Faculty of Sciences, Islamic Azad University Central Tehran Branch, Tehran, Iran.
Email: deli.hhamidi@yahoo.com

Background: Cryopreservation of spermatozoa is important because of new clinical demands. But sperm cryopreservation persuades serious changes in sperm function and sperm DNA integrity.
Materials and Methods: This experimental study was performed on 40 semen sample from normal and oligoasthenoteratozoospermia men. In all volunteers, after 3 days of sexual abstinence semen sample were collected by masturbation in sterile containers. After liquefication the sperm parameters were analysed according to World Health Organization (WHO). Each sample was divided into two groups: one of them is frozen with non-essential amino acid supplement, another without non-essential amino acid. After a while the samples were thawed. Then sperm parameters were analysed and DNA damage were assessed by TUNNEL assay based on protocol.
Results: This research showed that adding non-essential amino acid to cryopreserved semen can reduce DNA fragmentation index compared with control group.
Conclusion: Growing evidences suggested that some antioxidant like vitamins or amino acid can reduce the effect of ROS-induced and cold shock damage.
Key words: *Cryopreservation, Sperm, DNA fragmentation, Amino acid, Tunnel assay.*

P-23

The effect of vitamin C on gene expression of protamine 1 and 2 ejaculated sperm in male partners of couples experiencing recurrent spontaneous abortion

Hamidian S[1], Talebi AR[2], Mohammadzadeh M[2], Bayat M[1], Mirjalili S[2], Rajabi M, Montazeri F[4], Babaei S[1].
1. *Department of Anatomy, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Genetics Department of Biology, Yazd Science and Arts University, Yazd, Iran.*
4. *Abortion Research center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: saeid.babaei@yahoo.com

Background: Recurrent pregnancy loss (RPL) is defined as the miscarriage of two or more consecutive pregnancies in the first or early second trimester of gestation. Some study presents sperm abnormalities as one of the reasons for RPL. The sperm DNA and chromatin anomalies can affect embryo development and implantation. Antioxidants like Vitamin C protect protamine genes in sperm chromatin.
Objective: The aim of this study was to evaluate the effect of vitamin c on sperm chromatin and protamine genes expression in men of couples with a history of recurrent embryo loss.
Materials and Methods: In this randomized clinical trial, totally 40 men referred to the Reproductive Sciences Institute, Yazd were divided into 2 groups, including fertility control (n=20) and case with 2 or 3 RPL which were treated by Vitamin C (250 milligrams for 3 months). Sperm chromatin, DNA integrity using CMA3 and TUNEL Respectively and the ratio of the gene expression of protamine 1 and 2 (p1, p2) to compare with gene B2M (internal control) were assessed. Data analysis was done by to ANOVA assay.
Results: According to results, there were statistically significant differences between case and control groups and also between before and after vitamin administration regard to sperm morphology, the percentage of sperm cells with protamine deficiency and percentage of apoptotic cells ($p<0.05$). We saw a difference increase between before and after treatment of Vitamin regard to protamine genes expression ratios Respectively 3/9, 4/6. Also, Count sperm increased after vitamin administration.
Conclusion: According to our results, it seems that daily oral administration of Vitamin C through the increase of protamin gene expression improves, sperm parameter and DNA integrity in male partners of couples with RPL.
Key words: *Sperm, Recurrent pregnancy loss, Vitamin C, Protein gene, DNA fragmentation.*

P-24

Sperm parameters, DNA integrity, and protamine genes expression, in patients with diabetes mellitus

Imani M[1], Talebi AR[2], Fesahat F[3], Rahiminia T[4], Seifati SM[1], Dehghanpour F[2].
1. *Medical Biotechnology Research Center, Islamic Azad University, Ashkezar Branch, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4. *Gametogenesis Research Center, Fertility and Infertility Center, Kashan University of Medical Sciences, Kashan, Iran.*

Email: prof_talebi@hotmail.com

Background: Diabetes Mellitus (DM) is a chronic metabolic disorder and the most common endocrine disorder that affects many human physiological systems and tissues, including the reproductive organs in men,

while the age of individual suffering this disease has been falling rapidly in recent years.
Objective: The objective was to investigate the effect of DM on sperm parameters, chromatin quality, apoptosis as well as protamine genes expression profile in men with DM compared to controls.
Materials and Methods: Sixty semen samples from non-diabetic controls (n=30) and diabetic case groups (n=30) were compared for sperm parameters, protamine's transcripts and chromatin/DNA integrity using molecular and cytochemical assays.
Results: The results showed remarkable lower percentages of sperm parameters in cases versus controls ($p<0.05$). Despite the significant higher percentages of spermatozoa with AB+, CMA3+, and TUNEL+, no change was demonstrated regarding the protamine's mRNA levels as well as P1/P2 ratios in cases than in controls. In contrast, remarkable positive correlations were demonstrated between the quantity of P1 and P2 transcripts ($p<0.001$).
Conclusion: The data indicated DM not only causes a decrease in the quality of the sperm parameters but also affects the sperm maturation process by increasing the substantial implications in sperm DNA/chromatin levels of DM patients than normozoospermic controls.
Key words: *Sperm, Protamine deficiency, Gene expression, Male Infertility, Non-Insulin-Dependent Diabetes Mellitus.*

P-25
Sperm HSP90 transcript content in normo- and asthenozoospermic men

Izadi F[1], Dorostghoal M[1], Galehdari H[2], Hamadi M[3].
1.*Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, Iran.*
2.*Department of Genetic, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*
3.*Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
Email: izadifa1371@gmail.com

Background: There is a complex of mRNA transcripts in ejaculated sperm that participate in many cellular processes.
Objective: Present study was done to evaluate mRNA content of HSP90 in ejaculated sperm of normozoospermic and asthnozoospermic men.
Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University, Ahvaz, Iran. Sperm parameters were analyzed according to the World Health Organization (WHO) guidelines in men attending an infertility clinic and categorized to normozoospermic and asthnozoospermic groups. Real-time PCR was carried out to evaluate mRNA content of HSP90 in ejaculated sperm.
Results: Sperm content of HSP90 transcript was significantly ($p<0.05$) higher in asthnozoospermic than normozospermic men. Significant ($r=-578$, $p<0.01$) correlation was seen between sperm mRNA content of HSP90 and sperm motility.
Conclusion: These findings show that sperm mRNA content of HSP90 has important clinical significance and could be considered as a diagnostic tool in prediction of male infertility.
Key words: *Semen quality, Asthenozoospermia, HSP90, Transcriptome.*

P-26
Evaluation of *TNP1* gene and the neighboring lncRNA (lnc-AC007557) expression levels in the testicular tissue from azoospermic men

Katoeizadeh S[1, 2], Sanei Ataabadi N[2, 3], Nasr-Esfahani MH[2], Dormiani K[2].
1.*Department of Biology, Faculty of Science and Technology, ACECR Institute of Higher Education (Isfahan), Isfahan, Iran.*
2.*Department of Molecular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.*
3.*Department of Genetics, Faculty of Bioscience, Tarbiat Modares university, Tehran, Iran.*
Email: dormiani@yahoo.com

Background: Azoospermia is one of the causes of infertility in 10-15% of men and refers to the complete absence of sperm in ejaculate. Azoospermia divided into two major categories: Obstructive azoospermia (OA) and Non-Obstructive azoospermia (NOA). The TP1 protein plays an important role in the chromatin compression process during sperm maturation. During this process, first, TP1 replaces histones and then it is replaced by protamins. Long non-coding RNAs (lncRNAs) play significant role in regulation of gene expression in cis or trans-manner. Finding the lncRNAs related to NOA azoospermia genes may provide valuable insight into NOA azoospermia molecular mechanisms. On the other hand, lncRNAs are more specifically expressed in tissues and cells than coding genes, so they could be considered as suitable biomarkers for diagnosis of the infertility diseases.
Objective: In the present study, we aimed to evaluate the expression patterns of lncRNA AC007557 (LINC01921), placed in neighboring of *TNP1* gene (<10 kb). For this, the expression pattern of the TP1 was determined in NOA azoospermia using testicular biopsy of azoospermic patients. Besides, we assessed relationship between expression pattern of LINC01921 and *TNP1* gene.
Materials and Methods: 51 azoospermia testis tissue samples were collected from 36 Non-Obstructive azospermia (NOA) and 15 Obstructive azoospermia (OA) (as controls) from azoospermic men, referred to the Isfahan Fertility and Infertility Center for micro-TESE operation. They were classified histologically. Total RNA was extracted from tissue samples by TRIZOL regent and used for cDNA synthesis. The expression patterns of *TNP1* and LINC01921 were evaluated using Real-Time PCR and analyzed with $\Delta\Delta$Ct method. The biomarker potential efficiency of each gene was evaluated by Receiver operating characteristic (ROC) curve.
Results: The expression levels of *TNP1* and its neighboring lncRNA, LINC01921, were significantly decreased in NOA samples compared to OAs. In addition, there was a significant positive correlation

between *TNP1* and LINC01921 expression pattern. ROC curve analysis also showed area under the curve of 0.92 and 0.83 for *TNP1* and LINC01921 respectively, which confirmed their potential as azoospermia biomarkers.
Conclusion: In this study, the expression levels of LINC01921 and *TNP1* are evaluated in NOA samples as test and OA samples as control group. Our results demonstrated a significantly difference of expression level between two groups. Identification of neighboring lncRNA to the azoospermia gene can provide a valuable insight into male infertility and helps to detailed clarification of the molecular mechanism of the disease.
Key words: *lncRNA, TNP1, Azoospermia, Male infertility.*

P-27
Study of side effects of biolozax-H on testis tissue and epididymal sperm of adult rat following treatment with clinical dose

Kouhestanian K, Khayat Zadeh J, Zafarbalanezhad S.
Islamic Azad University, Mashhad Branch, Mashhad, Iran.
Email: kian.dokht@yahoo.com

Background: The activity of the renin-angiotensin system has an effect on oxidative stress in many tissues, including the testes. Therefore, in this study, the biolozax-H drug, which has an effect on the spermatogenesis activity and sperm quality in male rats, is further investigated.
Objective: The aim of this study was to assess side effects biolozax-H dose of biolozax-H on testis and epididymal sperm of adult male rat.
Materials and Methods: Male adult rats (200-250 gr) were divided into two groups of sixteen each. Control and biolozax-H treated group were administered 20 mg/kg biolozax-H for 45 days, respectively. The animals were sacrificed 45 days after starting treatment. The histological change on germinal epithelium, sperm quality parameters (count and normal morphology), viability and DNA fragmentation on sperm were analyzed by light microscopy, CASA (Computer-aided Sperm Analyzer).
Results: Biolozax-H administration significantly decreased parameters of sperm (count and normal morphology) and increased germinal epithelium destruction. The head, mid piece and tail abnormalities of treated group were increased significantly versus control. Higher levels of TUNEL positive cells that were found in treated groups demonstrated the increasing of DNA fragmentation in sperms following biolozax-H treatment.
Conclusion: The results showed that clinical dose of biolozax-H induces genotoxic, pre-apoptotic and cytotoxic effects on spermatogenesis. Also, biolozax-H can increase cytotoxicity on testis tissue of adult male rat.
Key words: *biolozax-H, Epididymal sperm, Sperm viability, Testis.*

P-28
Wnt signaling pathway in uterus of normal and seminal vesicle excised mated mice during pre-implantation window

Latifi Z[1], Nouri M[2], Fattahi A[2], Roshangar L[3], Nejabati H[1], Ghasemzadeh A[2], Hamdi K[2].
1.*Department of Clinical Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran.*
2.*Department of Reproductive Biology, Tabriz University of Medical Sciences, Tabriz, Iran.*
3.*Department of Anatomical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.*
Email: zeinablatifi@yahoo.com

Background: The importance of seminal vesicle secretion and uterine Wnt signaling for uterus preparation and embryo implantation has been described.
Objective: The aim of this study was evaluating effect of seminal vesicle secretion on uterine Wnt signaling.
Materials and Methods: In this study, we evaluated the gene expression of Wnt ligands (Wnt4 and Wnt5a) and their corresponding receptors (Fzd2 and Fzd6) using qRT-PCR and active beta-catenin protein levels using western blotting in the uterine tissue of female mice mated with intact and seminal vesicle-excised (SVX) males during the pre-implantation window. We evaluated the association between these factors and implantation rates and embryo spacing.
Results: mRNA expression of Wnt4 and Wnt5a and active beta-catenin protein levels decreased from Day 1 to Day 4, but reached a peak on the fifth day of pregnancy. Fzd2 also reached its highest level on Day 5. Fzd6 expression showed a decreasing trend towards the day of implantation. Lack of seminal vesicle secretion decreased Wnt4 and Wnt5a expression on Days 1 and 5 and beta-catenin levels on Day 5. There were almost no significant differences in expression levels of the Fzd2 and Fzd6 receptors between groups. There were positive and negative correlations, respectively, between implantation rates and embryo spacing and Wnt4, Wnt5a and active beta-catenin in the control group, but such correlations were not observed in the SVX-mated mice.
Conclusion: Significant changes occurred in the expression of several Wnt signaling members and there was a significant association between Wnt signaling and embryo implantation. Seminal vesicle secretion affects Wnt signaling in mice and consequently also affects murine embryo implantation.
Key words: *Wnt Signaling, Seminal vesicle, Embryo implantation.*

P-29
The protective effects of long term NaHS administration on sperm parameters, testicular morphometry, testicular H2S levels and oxidative stress markers in varicocele male rats

Lorian K.
Department of Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Email: klorian@razi.tums.ac.ir

Background: Varicocele, is characterized by abnormal dilation and tortuosity of veins of the pampiniform plexus. Hydrogen sulfide (H2S), an endogenous gaseous signaling molecule, has various anti-oxidant and

inflammatory functions in different parts of body. The aim of the current study was to evaluate the protective effects of NaHS (as a donor of H2S) on sperm parameters, testicular morphometry and testicular H2S levels and oxidative stress markers in varicocele male rats.

Objective: Varicocele, is characterized by abnormal dilation and tortuosity of veins of the pampiniform plexus. Hydrogen sulfide (H2S), an endogenous gaseous signaling molecule, has various anti-oxidant and inflammatory functions in different parts of body. The aim of the current study was to evaluate the protective effects of NaHS (as a donor of H2S) on sperm parameters, testicular morphometry and testicular H2S levels and oxidative stress markers in varicocele male rats.

Materials and Methods: Eighteen rats were randomly assigned to 3 experimental groups: 1) sham 2) varicocele 3) varicocele + sodium hydrosulfide. The sham group underwent sham operation and experimental groups were underwent partial ligation of the renal vein to induce experimental varicocele. Animals in varicocele + sodium hydrogen sulfide group received 30 µmol/l NaHS in drinking water for 8 wk. After 8 wk in all rats, caudal epididymis was used for collecting sperms in order to assess sperm parameters (motility and count). The left testis was excised, dissected free of surrounding tissue and was frozen immediately for measuring oxidative stress markers (MDA level and SOD activity) and testicular H2S levels. Testicular morphometry (tubule diameter and thickness) was assessed by Motic Plus Image.

Results: Varicocele caused significant decrease in sperm parameters (motility and count), oxidative stress markers (increased testicular MDA levels and decreased testicular SOD activity), decreased testicular H2S levels and testicular morphometry compared to sham group. NaHS administration improved sperm parameters, testicular morphometry, oxidative stress markers (decreased testicular MDA levels and increased testicular SOD activity) and elevated testicular H2S levels compared to varicocele group.

Conclusion: This study revealed that reduction in testicular H2S is a contributor factor in varicocele. Protective effects of NaHS administration were observed in current study. This treatment may be a promising strategy for protection against varicocele-induced male infertility in clinical practice.

Key words: *Varicocele, H2S, NaHS, Oxidative stress.*

P-30

Evaluation of semen sampling container toxicity with human sperm motility assay and mouse embryo assay

Maleki B, Izadi M, Mahaldashtian M, Tofighi M, Anbari F, Khalili MA.

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

Email: Khalili59@hotmail.com

Background: To improve the success rate in the ART lab, all consumables material and containers should be evaluated in quality control aspect. In this regard, two methods, Human sperm motility assay (HAS) and mouse embryo assay (MEA) are used for assessment of semen sampling containers toxicity.

Objective: Assessment of semen sampling containers toxicity with Human sperm motility assay and mouse embryo assay.

Materials and Methods: In this study, both MEA and HSA tests were used to evaluate the toxicity of sperm sampling containers. To carry out the HSA test, after the swim up of the normozoospermic samples (which had a motility more than 50%) was subjected to sampling containers for 1 hour. Both control and experimental groups were evaluated after 30 minutes, 1, 2, 4 and 24 hours. in each group, the percentage of sperm motility was evaluated. The ration of sperm motility in the test group to the control group was considered as an indicator for toxicity. To carry out the MEA test, a GLOBAL culture medium, commonly used in the ART laboratory for embryo culture, was exposed to a sperm sample container for 1 hour. Then, the medium was transferred to the embryo culture dishes. In both experimental and control groups, Blastocyst formation was assessed after 24, 48, 72 and 96 hr. Both tests were performed triplicate.

Results: The results of HSA test showed that the toxicity of the experimental group decreased significantly after 24 hours compared to the control group, while the results from MEA test showed that the toxicity level in the experimental group was not significantly different compared to control group.

Conclusion: It seems that using semen sampling containers has less toxicity for sperm and embryo.

Key words: *Semen sampling container, Toxicity, Human sperm motility assay, HSA, mouse embryo assay, MEA.*

P-31

Improvement of sperm parameters in old mice with low doses of estradiol and Sesame oil

Mohammadzadeh M[1], Farashahi-Yazd E[2], Motamedzadeh L[1], Anbari F[1], Nabi A[1], Ghasemi-Esmailabad S[1], Serpooshan AR[1], Khalili MA[1].

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

Email: khalili59@hotmail.com

Background: Aging led some changes in the male reproductive system, including reducing sperm parameters, increasing DNA deficiency. Consequently, the fertilization process is compromised. Studies have shown the use of exogenous estrogen solve some of the infertility problems. Also, the use of sesame oil as an antioxidant, protect sperm function from oxidative stress, as well as, use of antioxidant is recommended to improve sperm function.

Objective: Therefore, our aim of this study was achieved an appropriate dose of estradilo with sesame oil to optimize sperm parameters and chromatin.
Materials and Methods: Twenty-five old mice age range of 25-35 wk were divided into five groups: 1(control, without treatment), 2 (estradiol 100 µg/ml)), 3 (estradiol 10 µg/ml), 4 (estradiol 1 µg/ml) and 5 (Sesame oil). To each group, 10µl of treatment fluid was injected by IP method during 35 days, after that epididymal sperms were analyzed in terms of sperm parameters and chromatin and DNA status using Aniline Blue (AB), Tunel assay and Chromomycin A3 (CMA3) staining.
Results: The highest sperm motility belonged to group 3 ($E_2$10 µg/ml),. The lowest sperm count and motility were seen in groups 2 (E_2 100 µg/ml) this result was significant (p=0.004). Findings from sperm with arrest maturity was higher in high concentration than low concentration of estradiol and sesame oil therefore it has been shown a significant difference between groups (p=0.000). Sperm chromatin Integrity was a significant reduction in 100 µg/ml of estradiol but, in Sesame oil group and estradiol with 10 µg/ml, Sperm chromatin Integrity was more than other groups (p=0.037). In control and high concentration estradiol, the rate of DNA fragmentation was higher than in low concentration of estradiol (10 µg/ml) and Sesame oil but, there was no significant difference between groups.
Conclusion: This study showed that a low concentration of estradiol ($E_2$10 µg/ml) and Sesame oil improve the sperm parameters and chromatin Also, the Sesame oil as potent antioxidant had a better effect in low cicentration of estradiol than high concentrations.
Key words: *Estradiol, Old mice, Sperm parameters, Sesame oil, chromatin status, DNA integrity.*

P-32

Pentoxifylline increase sperm motility in devitrified spermatozoa from asthenozoospermic patient without damage chromatin and DNA integrity

Nabi A[1], Khalili MA[1], Fesahat F[2], Talebi AR[1], Ghasemi-Esmailabad S[3].
1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: khalili59@hotmail.com

Background: The freeze thaw process results in reduced motility, viability and fertilization potential of human spermatozoa. So, a variety of substances were evaluated in order to enhance human sperm resistance to the stress of cryopreservation, such as Pentoxifylline (PTX) for improving the Intracytoplasmic sperm injection (ICSI) outcomes.
Objective: The aim was to investigate the effect of PTX on sperm parameters and chromatin/DNA integrity of asthenozoospermic semen post vitrification.
Materials and Methods: A total of 30 semen specimens were obtained from infertile men with asthenozoospermia. The cryoprotectant-free vitrification was performed for the samples after assessment of sperm parameters. After warming, each sample was exposed for 30 min to 3.6 mmol/l PTX in experimental group and the control group without any treatment apposing at 37^{o}C for 30 min in regard, to repeat all in vitro analysis (sperm parameters and DNA integrity assay).
Results: Regardless of the vitrification devastating impacts on sperm parameters, incubation of post vitrified samples with PTX increased the rate of progressive motility ($p<0.01$). Moreover, PTX addition did not significantly damage DNA integrity of asthenozoospermic sperm samples.
Conclusion: The data showed that PTX was able to improve sperm movement without any adverse effects on sperm chromatin/DNA integrity in vitrification program.
Key words: *Pentoxifylline, Vitrification, Asthenozoospermia, DNA integrity.*

P-33

The ratio of Protamine 1 to Protamine 2 gene expression in sperm chromatin of varicoceles

Nayeri M[1], Talebi AR[2], Seifati SM[1], Tabibnejad N[2].
1.*Medical Biotechnology Research Center, Islamic Azad University, Ashkezar Branch, Ashkezar, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: prof_talebi@hotmail.com

Background: Varicocele or disturbance of testicular blood circulation is considered of one of the main cause of male infertility with decrease of sperm fertility potential. Although, there are many studies on the effects of varicocele on sperm parameters and some functional capabilities of these cells, but there are few data on sperm chromatin molecular changes in these patients.
Objective: The aim of present study is the evaluation of sperm chromatin/DNA integrity and the changes of protamine (1 & 2) genes expression in varicoceles.
Materials and Methods: 25 sperm samples as patient group and 25 sperm samples as control group were evaluated at first for sperm parameters according to WHO criteria. For sperm chromatin and DNA integrity, the CMA3 staining and TUNEL assay were done respectively. To examine the sperm protamine (1 & 2) expression, after RNA extraction and preparing of cDNA, the levels of protamines mRNAs were determined by RT-PCR method.
Results: The results showed that the sperm parameters significantly decreased in varicocele patients when compared to controls. In sperm chromatin condensation, the percentage of spermatozoa with protamine deficiency was greater than control ones. In the results of TUNEL assay, the rate of apoptotic spermatozoa showed

significantly increase in comparison with control fertile men. In molecular evaluations, we showed that the level of protamine 1 expression decreased in patients group, but the level of protamine 2 expression didn't show any significant difference with control group. It should be noted that the ratio of protamine was changed to about 1/2 of the ratio in control group.

Conclusion: Our results showed that in the case of varicocele, not only we have a reduction in sperm parameters, the rates of sperm chromatin condensation will decrease and sperm apoptosis will increase. On the other hand, the level of sperm protamine expression changes and so we can use this, as a good marker of male infertility due to the varicocele.

Key words: *Varicocele, Sperm, Chromatin, Protamine expression.*

P-34

Assessment of beneficial effects of methionine loaded zinc oxide nanoparticles on mice semen quality

Nazarian N[1], Nazem MN[1], Azizi S[2], Tavalaee M[3], Asadabadi Safaat A[4], Mohammadi P[5].

1.*Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
2.*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
3.*Biology Iranian Academic Center for Education, Culture and Research, Isfahan, Iran.*
4.*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
5.*Department of Chemistry, Shahid Bahonar University, Kerman, Iran.*

Email: Ehsan_Sakhaee@yahoo.com

Background: The extensive use of different nanoparticles has raised great concerns about their biological safety.

Objective: The aim of present study was to evaluate the effects of methionine loaded zinc oxide nanoparticles on mice semen quality.

Materials and Methods: Animals: Fifty sexually mature male NMRI mice weighed 25-30 gr (1.5-2 months old) were randomly divided into 5 groups with 10 animals and housed in cages at 21±2°C in an artificial dark-light (12 hr) cycle. During the study, the animals received water and pellet food (Javaneh Khorasan Co, Iran) ad libitum. Experimental design: Fifty mice randomly divided into 5 different groups (n=10/each): groups M50 and M100, which received 0.2 ml L-Methionin (Merck, Germany), diluted in distilled water, at dose of 50 and 100 mg/kg^{-1} daily for 6 wk, respectively. Groups N50 and N100 which received 0.2 ml L-Methionin loaded mesoporous zinc nanoparticles at dose of 50 and 100 mg/kg^{-1} daily for 42 days, respectively. Finally, group C (control) which received the same volume of normal saline. The supplements were injected intra-peritoneally in all animals, during experiment. Sperm quality analysis: Sperm samples were obtained from each group at the end of 6th wk. Samples of mature sperm were collected from the cauda region of epididymis by mincing it finely in PBS at 37°C. Sperm quality was determined by some parameters: Sperm concentration, motility and vitality. Sperm DNA damage was expressed as the DNA fragmentation index using Acridine orange staining, percentage chromatin condensation was assessed by Aniline blue, protamine deficiency in sperm chromatin was evaluated by Chromomycin and the percentage of sperm with high mitochondrial transmembrane potential was determined by Flow cytometry.

Results: Obtained results revealed that methionine loaded zinc oxide nanoparticles may have some beneficial effects on mice semen quality.

Conclusion: In conclusion, methionine loaded zinc oxide nanoparticles exhibited markedly different efficiencies in safeguarding spermatological parameters in the current study.

Key words: *Methionine, Mice, Sperm, Zinc oxide nanoparticles.*

P-35

Histomorphometric evaluation of mice testicular tissue following administration of Methionine loaded zinc oxide nanoparticles

Nazarian N[1], Nazem MN[1], Azizi S[2], Tavalaee M[3], Asadabadi Safaat A[4], Mohammadi P[5], Sheybani H[5].

1.*Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
2.*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
3.*Biology Iranian Academic Center for Education, Culture and Research, Isfahan, Iran.*
4.*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
5.*Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran.*

Email: Ehsan_Sakhaee@yahoo.com

Background: Nanoparticles have widespread use in medicine and related applications. Studies showed that nanoparticles have probable effects on male reproductive system.

Objective: The aim of present study was to evaluate the mice testicular tissue following administration of Methionine loaded zinc oxide nanoparticles.

Materials and Methods: Animals: Fifty sexually mature male NMRI mice weighed 25–30 g (1.5-2 months old) were randomly divided into 5 groups with 10 animals and housed in cages at 21±2°C in an artificial dark-light (12 hr) cycle. During the study, the animals received water and pellet food (Javaneh Khorasan Co, Iran) ad libitum. Experimental design: Fifty mice randomly divided into 5 different groups of 10 as follows: groups M50 and M100, which received 0.2 ml L-Methionin (Merck, Germany), diluted in distilled water, at dose of 50 and 100 mg.kg-1 daily for 6 wk, respectively. Groups N50 and N100 which received 0.2 ml L-Methionin loaded mesoporous zinc nanoparticles at dose of 50 and 100 mg.kg-1 daily for 42 days, respectively. Finally, group C (control)

which received the same volume of normal saline. The supplements were injected intra-peritoneally in all animals, during experiment. Histomorphometrical assays: After necropsy, the testis samples from all the animals of each group were preserved in 10% neutral buffered formalin (Merck, Germany) solution for histological examination at week 6. Formalin-fixed samples were processed by the standard paraffin wax technique, and sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E) and rate of spermatogenesis, meiotic index, diameter of seminiferous tubules, epithelium height of seminiferous tubules, and Johnsen scores determined.

Results: Results of the present study showed that there are significant differences between histomorphometrical characteristics of methionine loaded zinc oxide nanoparticles and control and methionine group.

Conclusion: It seems that it would be better to replace methionine with methionine loaded zinc oxide nanoparticles to improve testicular tissue safety.

Key words: *Testis, Histomorphometry, Methionine loaded zinc oxide nanoparticles, Mice.*

P-36

The effects of lycopene supplement on the spermatogram and seminal oxidative stress in infertile men; A randomized, double-blind, placebo-controlled clinical trial

Nouri M[1, 2], Amani R[1, 2], Nasr-Esfahani MH[2], Tarahi MJ[3].

1. *Department of Clinical Nutrition, School of Nutrition and Food Science, Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Departmen of Reproductive Biotechnology, Reproductive Biomedicine Research Centre, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.*
3. *Department of Epidemiology, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: rezaamani@hotmail.com

Background: Infertility is a major worldwide problem which is affected by several factors such as environmental, physiological, and genetic conditions. Some studies have shown a correlation between male infertility and reactive oxygen species (ROS) levels. Moreover, it was noted in several studies that the level of antioxidants and the antioxidant capacity in seminal fluid in infertile people is lower than usual. Lycopene is an aliphatic hydrocarbon that acts as an antioxidant defense against lipid peroxidation. Few studies have shown that lycopene may improve male infertility by increasing the antioxidant capacity of sperm.

Objective: The aim of this study is to evaluate the effect of lycopene supplementation on spermatogram and seminal oxidative stress.

Materials and Methods: In this randomized, double-blind, placebo-controlled trial study included 44 infertile men with oligozoospermia who were randomly divided into two groups, the experimental group was supplemented with 25 mg lycopene once a day and the control group received placebo for 12 weeks. Anthropometric, physical activity and dietary assessment, semen analysis, total antioxidant capacity (TAC), malondialdehyde (MDA) and glutathione peroxidase (GPx) were measured before and after the study. Independent t-test was used to analyze the initial variables, diet, sperm parameters, and oxidative stress biomarkers in the two groups. Paired t-test was used to compare the variables at the beginning and the end of intervention in each group. $P<0.05$ was considered as significant.

Results: At the end, 38 subjects completed the study: 19 in the lycopene group and 19 in the placebo group. There was no significant difference in weight, body mass index, body fat and physical activity at the end of the study in either groups. Mean energy and carbohydrate intake in both groups were significant at the beginning and the end of the study. However, protein, fat, and lycopene intake were not significantly different before and after the intervention. At the end of the study, there was a significant increase in total sperm count and concentration of sperm in the lycopene group compared with the placebo group, and after adjustment, it was also significant. Also, the within-group analysis showed a significant increase in ejaculate volume, total sperm count, concentration and non-progressive motion in lycopene group. TAC level changes in the two groups were significant, and after adjustment it was also significant. Also, Within-group analysis showed a significant increase in TAC level.

Conclusion: According to the current study, lycopene supplement could improve sperm parameters and oxidative stress biomarkers. However, further investigation is suggested in this regard.

Key words: *Lycopene, Infertile men, Oligozoospermia, Spermatogram, Oxidative stress.*

P-37

Effect of diet contains Sesame seed on adult Wistar rat testis

Ostovar N[1], Amini Mahabadi J[2], Hassani Bafrani H[2], Nikzad H[2, 3].

1. *Student Research Committee Kashan University of Medical Sciences, Kashan, Iran.*
2. *Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
3. *Anatomical Research Center, Kashan University of Medical Sciences, Kashan, Iran.*

Email: hhassanib@gmail.com

Background: Studies show that some antioxidants are effective in improving male infertility. According to several antioxidant compounds that exist in sesame seed.

Objective: This study was designed and carried out to the effects of sesame seed diet consumption on adult male rats testis structure and sex hormones.

Materials and Methods: This experimental study was carried out on 30 adults Wistar rat, 200 g that obtained from laboratory animal center at Kashan University of Medical Sciences. Rats were divided into experimental and control groups randomly. The control group received

standard diet and experimental group received diet containing 70% standard diet and 30% sesame seed after weaning for 12 weeks. At the end of the study, testis weight and volume were measured and seminiferous tubules; lumen epithelium diameter, LH, FSH and testosterone concentrations were evaluated. Data was analyzed by SPSS software and t-test. P<0.05 was considered to significant level.

Results: Bodyweight rats, weight and volume testis and percentage volume seminiferous tubules vessels in two groups were not significant. The mean cells number and motility of sperm in left epididymis, number of cells epithelium and percentage volume of epithelial, lumen and interstitial of this tubules were extremely significant (p<0.0001) in the experimental group compared to control. LH concentration increased significantly in the experimental group compared to control (p<0.03).

Conclusion: Sesame seed intake improved testicular parameters, fertility and sperm production in males.

Key words: *Sesame seed, Testis, Rat, Sex hormones.*

P-38

Sperm epigenetic features and a methyl donor: a randomized control trial study

Rahiminia T, Talebi AR.

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

Email: prof_talebi@ssu.ac.ir

Background: Folic acid is an important intermediary in the transfer of methyl groups in the synthesis of DNA, stability and integrity. But, the administration of this has not been investigated with the approach to improve the status of methylation in patients with sperm disorders.

Objective: Therefore, the aim of this study was to determine the optimization of sperm parameters and chromatin parameters, global DNA methylation level of sperm genomes and improvement the transcription retention of DNA methyltransferases (DNMTs).

Materials and Methods: This study is a randomized, controlled clinical trial. A number of 30 subjects with normal parameters were studied as the control group. Also, 30 subjects were selected for sperm analysis with parameters less than WHO criteria and randomly divided to two intervention treatment. In the two-drug group, vitamin E was given at a dose of 400 mg and selenium 200 mg daily and in the three-drug group, vitamin E 400 mg, selenium 200 mg and folic acid at a dose of 5 mg were prescribed daily (n=15/each group). After three months like before intervention, cytochemical tests were performed to evaluate the quality of chromatin and DNA of the sperm. Samples were compared using qRT PCR for DNMT1, 3A and 3B expression. The percentage of global DNA methylation was also evaluated based on ELISA.

Results: Sperm parameters such as concentration, progressive movement, normal morphology and viability were significantly higher in the three-drug group. The sperm decondensation and fragmentation of sperm DNA in the three-drug group was significantly lower than that of two-drug group. The global methylation of sperm DNA in the three-drug group was significantly reduced compared to pre- intervention. The increased global methylation was associated with a reduction in sperm parameters and chromatin integrity. The expression of DNMTs was not affected by the intervention.

Conclusion: The use of folic acid was able to improve sperm quality and epigenetic features in terms of decreasing the status of global methylation, increasing the chromatin and DNA integrity and also improving the sperm parameters.

Key words: *Methyltransferases expression, Sperm, Global methylation, Chromatin.*

P-39

Relation of sperm protamine transcripts content with global DNA methylation and DNA methyltransferases mRNA in men with severe sperm abnormalities

Rahiminia T[1], Farashahi-Yazd E[2], Hassani Bafrani H[1], Amini Mahabadi J[1], Talebi AR[3].

1. *Gametogenesis Research Center, Fertility and Infertility Center, Kashan University of Medical Sciences, Kashan, Iran.*
2. *Stem Cell Biology Research Centre, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Research and Clinical Centre for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: prof_talebi@ssu.ac.ir

Background: This study aims at evaluating the relation of mRNA expression of DNA methyltransferases (DNMTs) like DNMT1, DNMT3A and DNMT3B mRNA and sperm global DNA methylation with protamine transcripts content in men with severe sperm abnormalities.

Materials and Methods: Each semen sample was isolated and purified using a standard gradient isolation procedure issued by the WHO by layering 1 mL of 40% (v/v) density gradient mediume over 1 mL of 80% (v/v). The number of 30 oligoasthenoteratozoospermic patients (OAT) and 30 cases of normozoospermia as control were compared using real-time quantitative reverse transcriptase polymerase chain reaction for mRNA expression of DNMT1, 3A, 3B, protamine1 (P1) and protamine2 (P2). The enzyme-linked immunosorbent assay was used to detect sperm global DNA methylation. p<0.05 was considered statistically significant.

Results: The mRNA expression of P1, P2 and P1plus P2 as the total protamine transcripts was significantly higher in OAT. However, the P1 to P2 mRNA as the protamine ratio was lower in OAT (0.27) compared to control group (0.51) which was significantly different (p<0.04). The median of global DNA methylation was (3.1%) in OAT and (2.0%) in control group to a significant difference (p<0.001). The mRNA expression of DNMT1 was not statistically different in two groups. The mRNA expression of DNMT3A and DNMT3B were higher in OAT compared to that in control group to a significant difference (p=0.04, p=0.03 respectively) .In OAT neither

of protamine content of total nor ratio was related to sperm parameters. However, in control group P1plus P2 mRNA was negatively correlated to sperm progressive motility at a significant level.
Conclusion: In OAT, the increasing level of DNMT3A, 3B mRNA, sperm global methylation, P1plus P2 mRNA and decreasing of P1 to P2 ratio were significantly seen. In OAT, the content of protamine transcript was not correlated with sperm parameters.
Key words: *Sperm, DNA, Transcription.*

P-40
ICSI outcome in infertile males with round headed sperm who referred to Royan institute

Sabbaghian M, Kakavand K, Sadighi Gilani MA.
Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: marjan.Sabbaghian@gmail.com

Background: Globozoospermia or round-headed sperm syndrome is a very rare type of teratozoospermia with an incidence of less than 0.1% in infertile men population. The disorder characterized by absent or severely malformed acrosome due to failure in acrosome biogenesis, lead to incapacity of sperm to fertilize the oocyte. ICSI seems to be the main treatment for these patients to overcome infertility.
Objective: The aim of this study was to evaluate ICSI outcome of infertile men with round-headed sperm referred to Royan institute.
Materials and Methods: This is a retrospective study on ICSI outcome of 163 infertile couples which male partner has more than 50% round head spermatozoa, referring to Royan institute (Tehran, Iran) between years 2008 and 2016.
Results: From these 163 patients, 53 couples underwent 66 ICSI cycles. The fertilization rate (FR) was 38%. Afterwards, 43 couples had a total number of 60 embryo transfer. 18 pregnancies out of 66 cycles achieved, showed a 27% pregnancy rate. 16 deliveries out of 66 cycles, 24% delivery rate, with 21 live births (15 male and 6 female) also have been achieved.
Conclusion: Regarding the current study results and previous reports, ICSI could be a hopeful plan for treating infertility caused by round-headed spermatozoa, at least until other effective and safe treatments will become available in future.
Key words: *Globozoospermia, Male infertility, ICSI, Acrosome.*

P-41
Effect of Carbamazepine and Oxcarbazapine on chromatin quality, Malondialdehyde and parameters in sperm of epileptic-mice

Tadayyon Nezhad M[1], Anvari M[1, 2], Talebi AR[1, 2], Pourentezari M[1], Rezvani M[3], Shahedi A[1].
1. *Department of Biology and Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences, Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Department of Physiology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: mahsa_tadayyon70@yahoo.com

Background: Consumption of anti-epilepsy drugs, recently, has become very common. These drugs have a clear effect on the reproductive system.
Objective: The study is aimed to assess the effects of carbamazepine (CBZ) and ox-carbazepin (OXC) on sperm parameters, chromatin quality, and DNA integrity and evaluate Malondialdehyde (MDA) in NMRI/ mice spermatozoa.
Materials and Methods: Thirty-two adult male NMRI mice were separated into four groups. Group1 (Control), received basal diet; group 2, pentylenetetrazol (PTZ) (35 mg/kg); group 3, PTZ (35 mg/kg) and CBZ (30 mg/kg); group 4, PTZ (35 mg/kg) and OXC (15 mg/kg). CBZ and OXC were administered by oral gavage and PTZ was given as intra peritoneal (IP) method every 48 hr for 35 days. Finally caudal epididymis was removed, and placed in 1 mL Ham'"sF10 medium at 37^{o}C for 15 min in incubator and then analysis of sperm parameters was executed. To asses sperm chromatin quality and DNA integrity, we use toluidine blue (TB), chromomycin A3 (CMA3), Aniline blue (AB) and TUNLE, and to observe lipid peroxidation and total antioxidant capability, the MDA were measured.
Results: The mean sperm parameters in the epileptic group, were significantly lower than the other groups. The sperm parameters in the groups treated with OXC and CBZ drugs were distinctly better than the epilepsy group and weaker than the control group, ($p<0.05$). In sperm chromatin quality and DNA integrity'"s test there were no significant different between group CBZ and OXC in AB, TB, CMA3, TUNLE, ($p<0.05$). But there were a significant different between groups CBZ and OXC with group PTZ, ($p<0.05$). There were a significant different between groups CBZ and OXC with control group in AB and TB test. MDA tests showed a significant difference between CBZ and OXC groups, and a significant different between CBZ and OXC groups with control and PTZ groups, ($p<0.05$).
Conclusion: The results of this study showed that epilepsy can weaken sperm parameters, which can be leading to the increased production of reactive oxygen species (ROS). The use of antiepileptic drugs slightly improves the parameters and the quality of the chromatin and DNA integrity of the sperm epileptic mice and the lipid peroxidation in this group.
Key words: *Carbamazepine, Oxcarbazepine, Sperm chromatin, Malondialdehyde, Mice.*

P-42
Relationship between sperm progressive motility and DNA fragmentation in fertile and infertile men

Torkiyan N.
Saint Maryam Infertility Center, Shahid Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran;
Email: nayerazam.torki.123@gmail.com

Background: Sperm progressive motility has been reported to be one of the key factors influencing in vitro and in vivo fertilization rates. There has been an increase in the literature of studies investigating whether DNA fragmentation could be associated with other semen parameters; however, few reports focused on the relationship between sperm DNA fragmentation and progressive sperm motility.
Objective: This study aimed to determine the relationship between DNA fragmentation level and progressive sperm motility in different groups of infertile asthenozoospermic patients as well as in healthy men of proven fertility.
Materials and Methods: Semen samples were collected and examined after liquefaction for 20 min at 37°C from patients in Andrology Laboratory of Majesty of Maryam Infertility Center, Martyr Beheshti Hospital, Isfahan, Iran. Patient were then classified as asthenozoospermic [(Mild asthenozoospermia; PR (progressive sperm motility) = 30-20% n=50) (Moderate asthenozoospermia; PR= 20-10% n=50) and (Severe asthenozoospermia; PR <10% n=50] and 50 fertile healthy men as a control.
Results: Fertile healthy men showed lower sperm DNA fragmentation levels as compared with asthenozoospermic infertile men. There was a significant negative correlation of sperm DNA fragmentation using the modified sperm chromatin dispersion (SCD) test with motility ($p<0.001$) and progressive motility ($p<0.001$).
Conclusion: Overall, our data suggest that sperm DNA damage is strongly associated with both type and percentage of motility.
Key words: *DNA fragmentation, Progressive motility, Male.*

P-43
Iranian temporal changes in semen quality during past 25 years: report from tertiary infertility center

Vahidi S[1], Moein MR[1], Yazdinejad F[1], Ghasemi-Esmailabad S[2], Narimani N[1].
1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: Nima_dr2001@yahoo.com

Background: Although there are numerous reports about temporal changes in semen quality from all over the world, debates continue. The latest systemic review observed an overtime decrease in semen quality worldwide. This is the first report of its kind from our country.
Objective: To assess the temporal changes of semen quality among Iranian population which was referred to a tertiary infertility center.
Materials and Methods: Through a retrospective study, we compared semen parameters of 707 Iranian male patients between 1990 to 1992 (group 1) with 1108 men from 2010 to 2012 (group 2).
Results: We showed despite increased sperm concentration from 84.48 in group 1 to 95.55 in group 2, sperm with normal morphology decreased significantly from 62.2 to 44.44%, grade A motility decreased significantly from 38.6% to 30.6%, grade B motility increased significantly from 21.34 to 30.3 % and grade C and D motile sperm remained constant. We also assessed the effect of age on semen parameters between 2 groups.
Conclusion: We observed inconsistent changes of semen quality over time. These results should be further evaluated by larger sample groups in the future.
Key words: *Infertility, Iranian, Temporal changes, Semen quality, Male factor.*

P-44
The effect of L-carnitine on sperm parameters in adults BALB/C mice treated with formalin

Vardiyan R, Ezati Yazdani D, Talebi AR, Anvari M.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: reyhanevardiyan546@gmail.com

Background: The effects of formaldehyde on fertility and reproductive system have been proven to indicate abnormalities in sperm and reduce fertility rates. According to research, L-carnitine is a therapeutic way in patients with azoospermia.
Objective: This study aimed to investigate the effect of L-carnitine on sperm parameters in BALB/C treated with formalin, as well as the effect of this drug as prophylaxis in people exposed to formalin.
Materials and Methods: In this interventional study, 32 adult male BALB/C mice (10-12 wk-old) with a weight of 30-35 gr were selected and randomly divided into 4 groups of 8, including: 1- The control group was kept for a period of 31 days without any injections and gavage. 2- Sham group, only normal saline serum of 10 ml/kg intraperitoneally for 31 days, the 3rd group was exposed to formalin, 10 mg/kg injected intraperitoneally and stored for 31 days. The 4th groups were exposed to formalin and L-carnitine, injected 10 mg/kg intraperitoneal daily and L-carnitine were dosed at 100 mg/kg for solution in water for up to 31 days. Then the rats were killed by spinal cord injury. The sperm samples were taken from the tail of epididymis and the sperm analysis was performed.
Results: This study showed that formalin administration in formalin group significantly reduced the testicular weight; sperm motility, sperm count, survival percentage and natural morphology in comparison with the control group ($p\leq0.05$). Sperm parameters in formalin and L-carnitine group significantly improved these parameters ($p\leq0.05$).
Conclusion: According to the results, it can be concluded that L-carnitine improves the effects of formalin exposure. L-carnitine may improve and protect mice sperm parameters treated with formaldehyde respectively so it can be used as a prophylaxis in these individuals.
Key words: *Formalin, L-carnitine, Sperm parameters, Male mice, Sperm count.*

P-45
The effect of buffering leasing on parameters, chromatin and human sperm DNA

Yazdinejad F, Dehghanpour F, Motamedzadeh L.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: 63rahimi@gmail.com

Background: Azoospermia may have a low sperm count during testicular biopsy, which is an invasive surgery. Finding this low number of sperm between blood cells is very important and by removing the blood cells, access to these sperms and their use during microinjection is easier. For this purpose, fluoridation that is used to remove blood cells should not affect sperm.
Objective: This study investigates the effects of this leasing buffer on sperm parameters, sperm survival, sperm morphology, chromatin, and sperm DNA.
Materials and Methods: A total of 23 samples of semen were collected and the amount of protamine deficiency was determined by staining CMA3 (chromosomal A3), the aniline blue level (AB), for anesthetic anesthetics, Toluidine blue (TB) chromatin anomalies and apoptosis with Tunnel (TUNEL) was evaluated and compared.
Results: As a results, there was no significant difference in the concentration of spermatogenesis mixed with beef rhealing compared to the pre-freezer group. Positive Tunnel sperms (TUNEL +) increased significantly in the spermatozoa that had been mixed with bifurylation compared to the other group.
Conclusion: Fluoridation does not affect the morphology and the low levels of prothromatin, reduces sperm motility and increases the level of chromosomal anomalies apoptozone.
Key words: *Lising buffer, Testicular sampling, Sperm morphology, Male infertility, Sperm chromatin, Apoptosis.*

P-46
Semen preparation methods and PLC-ζ expression

Yousefian E[1], Khosravi S[1], Allavaisie A[2], Bagheri N[1].
1.*Saint Maryam Infertility Center, Shahid Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
2.*Department of Anatomy, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*
Email: Elham.yousefian@med.mui.ac.ir

Background: Phospholipase C zeta (PLC-ζ), is considered as a biomarker of sperm fertilizing capacity. The PLC-ζ has been identified as a critical mediator of Oocyte activation. It is believed that some types of male infertility are caused by reduced protein expression levels and abnormal forms of PLC-ζ. The present study was designed to compare the effectiveness of gradient and swim-up, either alone or in combination to select sperm with higher PLC-ζ expression.
Objective: The present study was designed to compare the effectiveness of gradient and swim-up, either alone or in combination to select sperm with higher PLC-ζ expression.
Materials and Methods: A total of 30 subjects visiting the Andrology Unit of University infertility clinic with normozoospermic parameters oligozoospermia and teratozoospermia were included. Semen characteristics were analyzed by standard criteria. Then, PLC-ζ protein expression was assessed by quantitative Immunofluorescence in sperms after processing by gradient, swim-up, either alone or in combination.
Results: PLC-ζ content after sperm processing was significantly higher in normozoospermic ($p<0.05$), oligozoospermic ($p<0.001$) and teratozoospermic samples ($p<0.01$). Spermatozoa selected by 3 different methods were investigated and compared according to PLC-ζ content. DGC + swim-up combination significantly increased the proportion of spermatozoa with PLC-ζ expression ($p<0.05$). However, no significant difference was found between the DGC and swim-up procedures ($p>0.05$).
Conclusion: Sperm preparation by combination gradient and swim-up has been found to results in enrichment of sperm with higher PLC-ζ expression.
Key words: *Phospholipase C zeta, Semen preparation, Sperm.*

P-47
Identification of new mutations in mitochondrial ATPase6 and ATPase8 genes in asthenospermic infertile men and investigation of protein structure using molecular dynamics simulation

Zare-Dehghanani Z.
Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: zahrazare228@yahoo.com

Background: Infertility affects 10% and 15% of couples, accounting for about 50% of infertility related to male factor. Approximately 30% of male infertility factors are associated with genetic disorders such as a mutation in nuclear genes and mitochondria. Mitochondria in various forms affect infertility. The ability to move active flagella is important for the proper functioning of the sperm and the successful transmission of the female reproductive system. The mitochondria supply the energy of sperm through the oxidative phosphorylation through the electron transfer chain. However, mitochondria in the middle segment of sperm as the major source of intracellular reactive oxygen species and free radicals, leading to lipid peroxidation and the formation of reactive products such as malondialdehyde and ultimately leads to a reduction in fertility in men.
Objective: The aim of this study was to determine the genetic evaluation of mitochondrial ATPase6 and ATPase8 regions in asthenospermic infertile men and the association between mutations in these region with two biochemical markers of oxidative stress and to investigate the effect of known mutations on the structure of proteins encoded by these two genes for better understand the performance of these two protein subunits.

Materials and Methods: In this study, 100 samples of semen from the fertile and infertile men of asthenospermia from Infertility Center of Shahid Beheshti Hospital in Kashan were collected. The sperm cells were isolated from the semen by the swim up. The total antioxidant capacity and malondialdehyde plasma levels of the seminal were measured. After extraction of DNA from the semen, the mtATPase6 and ATpase8 region was amplified by PCR technique. By determining the sequence of this region in the patient samples, 43 nucleotide changes were reported. Then, using the PANTHER and polyphen-2 database, the effects of mutations were investigated. After modeling the desired protein, molecular dynamics simulation of two malignant mutations was performed to investigate the effect of structural changes in the protein.
Results: Our results showed that the average level of ATC of semen plasma in infertile men was significantly lower than that of fertile men, compared with malondialdehyde level of infertile men was significantly higher than fertile men. These results indicate a low sperm motility relation with electron transfer impairment, while simulation results indicate structural changes in proteins.
Conclusion: Therefore, clarifying the molecular basis of these defects in infertile men with asthenospermia with a history of unknown infertility is necessary for better diagnosis and management of infertility treatment.
Key words: *Asthenosperm, Mitochondrial DNA, ATPase6 gene, ATPase8 gene, Molecular dynamics.*

2- Embryology

P-48

The effect of endometrial injury on *VEGF* gene expression in patients with repeated implantation failure: A randomized control trial

Aghajanpour S[1], Amirchaghmaghi E[2], Ashrafi M[3], Aflatoonian R[1].
1.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2.*Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
3.*Shahid Akbarabadi Clinical Research Development Unit, Iran University of Medical Sciences, Tehran, Iran.*
Email: r.aflatoonian@gmail.com

Background: Human embryo implantation is mediated by a complex interaction between embryo and endometrium, which is necessary for successful pregnancy. The failure of implantation may be decreased endometrial receptivity, defective embryonic development or both of them. Repeated implantation failure (RIF) is an unknown major barrier of fertility in infertile women. It is assumed that a defective receptive endometrium is the main cause of RIF. Many strategies have been done to improve the implantation rate in RIF patients. Endometrial injury (EI) is one of these procedures which recently received more attention. However, the mechanisms of EI remains controversial. It seems EI can trigger angiogenesis which is an important aspect of successful implantation. On the other hand, vascular endothelial growth factor (VEGF), as an angiogenic factor, was closely involved in endometrial receptivity and embryo implantation.
Objective: The aim of this study was to determine the effects of EI in follicular phase on *VEGF* gene expression in patients suffering from RIF.
Materials and Methods: A total of twenty women with RIF who failed to conceive during two or more IVF/ICSI cycles and embryo transfer (ET) participated in this randomized controlled trial (RCT) study. Pipelle endometrial sampling was done twice: One in the follicular phase as endometrial injury and another in the luteal phase of same menstrual cycle in case group (n=10). Endometrial sampling was done once in control group (n=10) just in the luteal phase for genomic evaluation. Total RNA was extracted from endometrial tissues, then VEGF gene expression was investigated by quantitative real-time PCR.
Results: In this study we examined the effect of EI on *VEGF* gene expression. This study showed that VEGF was detected in endometrial samples of both groups in luteal phase. The mean relative expression of *VEGF* gene was statistically higher in the case group in comparison to control group at the same time.
Conclusion: As a whole, our study provided molecular evidence that patients with repeated implantation failure can benefit from local injury in follicular phase of menstrual cycle before ongoing IVF cycle.
Key words: *Endometrial injury, RIF, VEGF, Embryo Implantation.*

P-49

Taurine improves the function of mice ovary grafts through reducing oxidative stress

Ahmadi S, Soleimani Mehranjani M.
Department of Biology, Faculty of Science, Arak University, Arak, Iran.
Email: sepidehahmadi358@gmail.com

Background: Ovarian tissue transplantation is an experimental procedure for preserving fertility in cancerous patients facing reproductive function failures. ischemia/ reperfusion (IR) injury is one of the major limitations of ovary transplantation, which leads to the excessive production of reactive oxygen species (ROS). Taurine as a strong antioxidant is a suitable candidate for preventing I/R injury.
Objective: We aimed to investigate the effect of taurine on ovary function following autotransplantation.
Materials and Methods: The Naval Medical Research Institute (NMRI) mice (4-5 wks old) were divided into three groups: control; autograft +saline, and autograft +Taurine (i.p injections 200 mg/kg/day). Treatment was carried out one day before till 7 days after transplantation.

Seven days after ovary autografting, serum concentrations of malondialdehyde (MDA) and the total antioxidant capacity were estimated. 28 days post transplantation, serum concentrations of progesterone (P4) and estradiol (E2) were also evaluated. The results were analyzed using one-way ANOVA and Tukey's test and the means were considered significantly different at $p<0.05$.

Results: A significant decrease in the total antioxidant capacity, concentration of progesterone and estradiol was found in the autografted group compared to the control group ($p<0.001$). The Concentration of MDA significantly increased in the autografted group compared to the control group ($p<0.001$). The mentioned parameters were ameliorated to the control level in the autografted + Taurine group.

Conclusion: Our results indicated that the administration of taurine reduces oxidative stress, increases the total antioxidant capacity and restores the hormone levels in the grafted ovaries which can improve the function of the grafted ovary.

Key words: *Autotransplantation, Ovary function, Taurine, Mice.*

P-50
L-carnitine could suppress cyclophosphamide induced oocyte apoptosis in mice

Almasi M, Shafiei G, Nikzad H, Moshkdanian G.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: majid.almasi72@gmail.com

Background: With the advent of early cancer diagnosis and more effective individualized cancer treatments, a growing number of young cancer patients become long-term survivors during their reproductive years. Cyclophosphamide (CP) is one of the most effective alkylating agents that used for cancer therapy which can be mostly attributed to ovarian toxicity and depletion of the oocyte. One of the options for fertility preservation in female cancer patients uses Antioxidant. Antioxidant that can prevent the loss of follicles at the time of treatment would provide significant advantages over existing fertility preservation techniques in that they would be suitable for patients of all ages and life stages. L-carnitine (LC) acts as an antioxidant that neutralizes the free radicals especially superoxide anion and protects cell against oxidative damage. L-carnitine should be viewed as a leading candidate and must be given along cyclophosphamide to block their ovarian toxicities.

Objective: In our current study, we examined the antiapoptosis effect of L-carnitine on oocyte in Cyclophosphamide Treated Mice.

Materials and Methods: Female NMRI mice were divided into four groups (N=10): control group was intraperitoneally injected with normal saline for 10 consecutive days; group CP was an intraperitoneal injected single dose of Cyclophosphamide (75mg/kg); LC and CP+CL groups were injected with L-carnitine (200 mg/kg, i.p.) for 10 consecutive days and the CP+CL group was received single dose of Cyclophosphamide (75 mg/kg) On the tenth day. Female NMRI mice were injected with 8 IU pregnant mare serum gonadotropin (PMSG) followed by 8 IU human chorionic gonadotropin 48 hours later and then oocytes were retrieved 14 hours later. Then the Caspase3, BAX, and Bcl-2 expression were evaluated by Real-time PCR. Moreover, intracellular ROS level was examined by DCFH-DA fluorescence intensity.

Results: LC can enhance significantly ($p<0.05$) Bcl-2 gene expression and decrease Caspase3 and BAX genes expression in LC + CP group in comparison with the CP group. and also L-carnitine can significantly ($p<0.05$) decrease the mean of intracellular ROS in CP+LC group in comparison with CP group (28.77 vs. 42.58).

Conclusion: The results of our study showed the effective role of L-Carnitine in the removal of ROS produced in the oocytes encountered with the cyclophosphamide chemotherapy drug, therefore, L-carnitine can be used as an option to protect the oocytes against damage Induced by cyclophosphamide.

Key words: *L-carnitine, Cyclophosphamide, Oocyte, Apoptosis.*

P-51
Decreased expression of ADAMTS-9 and ADAMTS-5 associated with impaired oocyte maturity in PCOS patients

Artimani T[1], Gohari Taban S[2], Amiri I[1], Soleimani Asl S[3], Saidijam M[1], Yavangi M[1], Mohammadpour N[1], Afshar S[3], Mirzaee B[1].
1. *Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.*
2. *Department of Anatomy, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
3. *Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
Email: artimani@umsha.ac.ir

Background: Ovulation is a controlled event that precisely directs oocyte maturation, oocyte selection and rupture and oocyte releasing. More recently, evidence has emerged indicating an important role for members of newly described ADAMTS family of metalloproteinases during the periovulatory interval. PCOS is a common disease with failure in the selection of dominant follicle which results in anovulation. The role of ADAMTS-9 and ADAMTS-5 in the ovulation process is not yet known.

Objective: The aim of this study was to investigate changes of ADAMTS-9 and ADAMTS-5 gene expression in cumulus cells of PCOS and their association with progesterone receptors expression and oocyte Maturity.

Materials and Methods: After approval of Hamadan University of medical sciences ethics committee and written informed consent of the patient, CCs were isolated from 37 patients with PCOS (according to Rotterdam criteria) and 37 healthy women with normal ovarian function between 2016 and 2017. Purified CCs of patients were separately pooled to form the CCs in GV-stage and CCs in MII-stage groups. After ovarian

stimulation with a GnRH agonist, recombinant FSH, and HCG, oocytes were retrieved via ultrasound-guided vaginal puncture. Then CCs were isolated and categorized according to the oocyte nuclear maturation stage: GV or MII (metaphase II stage). Total RNA was extracted and reverse transcription was performed. Gene expression of ADAMTS-9 and ADAMTS-5 was determined by quantitative real-time PCR method (q-PCR). All statistical procedures were run on SPSS 16. $p \leq 0.05$ was considered significant.

Results: Our data revealed that ADAMTS-9 and ADAMTS-5 express in the human CCs. qRT-PCR demonstrated a decreased expression levels of ADAMTS-9 and ADAMTS-5 in the CCs of PCOS women compared to the healthy women with normal ovarian function ($p=0.01$ and $p=0.007$ respectively). Furthermore, we observed increased expression levels of ADAMTS-9 and ADAMTS-5 in the CCs of mature oocytes compared to the GV oocytes ($p=0.01$ and $p=0.001$ respectively). Recent studies confirmed a key role for ADAMTS-1 protease as a downstream effector of progesterone receptor function in ovulation. In this study, we observed a significant positive correlation between the expression of progesterone receptor B with ADAMTS-9 and ADAMTS-5 ($r=0.4$, $p=0.0006$, and $r=0.57$, $p=0.0001$ respectively).

Conclusion: ADAMTS-9 and ADAMTS-5 expression in GV oocytes were lower than mature ones. ADAMTS-1 and ADAMTS-9 were upregulated prior to the ovulation in the normal cycling rats and downregulated in PCOS CCs failed to progress through the meiosis. These findings help to further understand PCOS pathology and improve in vitro maturation outcome.

Key words: *ADAMTS-9, ADAMTS-5, PCOS, Cumulus cells, Oocyte Maturity.*

P-52

Associaton of androgen receptor CAG polymorphism with idiopathic male infertility

Barati E, Mobasseri N, Karimian M, Nikzad H.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: mdkarimian@gmail.com

Background: CAG repeats polymorphism in the androgen receptor (*AR*) gene may change the risk of male infertility.

Objective: The aim of this study was to investigate the association of AR-CAG repeats with male infertility.

Materials and Methods: In a case-control study, 150 fertile and 150 idiopathic infertile men were included. After blood sample collection and genomic DNA extraction, polymerase chain reaction (PCR) method with following the electrophoresis on polyacrylamide gel was used for AR-CAG genotyping.

Results: Our case-control study revealed that there is a significant association between an increase in the length of AR-CAG polymorphism and male infertility ($p<0.01$). Also, there were similar significant associations for azoospermia ($p<0.05$), asthenozoospermia ($p<0.05$) and teratozoospermia ($p<0.01$) subgroups.

Conclusion: Our results elucidate that long stretches of CAG repeat may lead to AR dysfunction and this may contribute to male infertility especially in the Caucasian population.

Key words: *Male infertility, Androgen receptor, CAG repeat, Gene polymorphism, Genetic association.*

P-53

Analysis of olfactory receptors expression in cumulus cells as predictor for maturation level and fertilization outcome of oocyte

Daei-Farshbaf N[1], Amjadi FS[1], Taleahmad S[2], Ashrafi M[1], Aghajanpour S[3], Bakhtiyari M[1], Aflatoonian R[3].
1. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2. *Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell, Tehran, Iran.*
3. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: r.aflatoonian@gmail.com

Background: Morphological changes during meiotic division are indicative of simultaneous cytoplasmic and nuclear maturation of oocyte which will determine its final quality and fertilization outcome. Although several molecules are involved in these phenomena, it has been proven that the calcium fluctuation plays vital role in oocyte maturation. There is evidence that mainly intercellular sources release their calcium, but extracellular calcium participation rate is less known.

Objective: We analyze the proteome profiling of cumulus cells (CCs), in order to discover proteins which support oocyte maturation and provide fertilization competency by managing extracellular calcium trafficking in CCs.

Materials and Methods: Proteomics were conducted on cumulus cells of oocytes at different maturation stages of metaphase I (MI), unfertilized metaphase II (Uf-MII) and fertilized metaphase II (F-MII). These cumulus cells were obtained from tubal factors and donors with normal oogenesis using GnRH agonists. Three-step analysis were performed: as the first step, proteomic data were analyzed, proteins were organized in related biological pathways, and olfactory transduction pathway was selected due to its role in calcium trafficking. Second, western blot and qRT PCR were conducted on olfactory marker protein (OMP) and adenylyl cyclase 3 (AC3) -as olfactory neuron biomarkers- proteins and genes respectively to confirm the function of olfactory pathway in cumulus cells. Since in several studies, it is demonstrated that olfactory receptors (ORs) have specified expression pattern in brain of a young and healthy person which could be altered due to ageing and some disorders such as Alzheimer and Parkinson diseases, in final step, eight olfactory receptors were selected from ORs expressed ectopically in cumulus cells

and after performing qRT PCR, special expression patterns of these 8 ORs were analyzed in all 3 groups.
Results: The results demonstrate increased expression of OMP and AC3 proteins and related genes in the cumulus cells of F-MII oocytes. Moreover, drawn patterns of selected ORs show that oocytes at different maturation stages, depending on their maturation level and fertilization potential, have specific expression pattern of ORs in related CCs.
Conclusion: The present study strongly supports a functional role of olfactory receptors in cytoplasmic and nuclear oocyte maturation by impacting on calcium trafficking. However, ectopic expression and specific pattern of ORs in human cumulus cells were confirmed by this study, but how ORs are regulated by gonadotropins or other factors remains to be determined.
Key words: *Cumulus cells, Proteomics, Olfactory receptors, Oocyte maturation, Calcium.*

P-54
Metabolome mapping of day 3 and 5 embryo culture medium using MALDI-TOF-MS

Ebrahimian N[1, 2], Sadeghi MR[3], Kalantar SM[2], Khalili MA[4], Gilany K[3].
1.*Reproductive Embryology and Andrology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research, Tehran, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Reproductive Immunology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research, Tehran, Iran.*
4.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: k.gilany@ari.ir

Background: The purpose of this study was to map secreted metabolome from embryo culture medium of Day 3 and 5.
Objective: The purpose of this study was to map secreted metabolome from embryo culture medium of day 3 and 5.
Materials and Methods: Embryo culture media was collected on day 3 and 5. Metabolome of pure culture medium (without embryo) and secreted metabolome of embryo culture medium Day 3 and 5 were extracted and analyzed by MALDI-TOF-MS. Metabolites were identified using The Human Metabolome Database-HMDB version 4.0. The Reactome Pathway Database was used for characterization of mapped metabolome of Day 3 and 5.
Results: 775 m/z peaks were detected. More than 400 metabolites were identified of secreted metabolome from Day 3 and 5 embryo culture medium using Human Metabolome Database. However, only 9 identified metabolites were in overlap between Day 3 and 5. Reactome analysis showed metabolite belonging to metabolism, signal transduction and transport of small molecule were enriched. However, the majority of metabolites biological activity remains unknown.
Conclusion: To the best of our knowledge this is the first metabolome mapping of embryo culture medium using MALDI-TOF-MS. Although, we mapped potential metabolome from Day 3 and 5 the function of majority of secreted identified metabolites remain unknown during embryo development. The potential embryo secreted metabolome from Day 3 and 5 can be found Supplementary material.
Key words: *Metabolome, MALDI-TOF-MS, Database-HMDB, mMetabolomics.*

P-55
Morphometric analysis of human oocytes does not predict embryos developmental outcomes: a time lapse study

Faramarzi A[1, 2], Khalili MA[2].
1.*Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: a.faramarzi90@gmail.com

Background: It is certain that the outcomes of intra-cytoplasmic sperm injection (ICSI) rely on the quality of selected oocytes for this procedure. Objective evaluation of oocyte quality is really a main issue of studying in assisted reproduction technology (ART) programs. Oocyte size and the quantitative properties of its components could suggest several clues about the oocyte quality.
Objective: The aim of this prospective study was to evaluate the relationship between the morphometric parameters of metaphase II (MII) oocytes and morphokinetic behaviors of derived embryos in ICSI setting.
Materials and Methods: The correlation of oocyte morphometry (whole oocyte), ooplasm, width of zona pellucid (ZP) and periviteline space (PVS), and first polar body (PB) with embryo morphokinetic variables including time of 2nd PB extrusion (tPB2), time of pronuclei appearance (tPN), time of pronuclei fading (tPNf), time of two to eight discrete cells (t2 to t8) and irregular cleavage events [uneven at 2 cells stage, cell fusion (Fu) and trichotomous mitoses (TM)] were assessed.
Results: Only tPB2, t5 and t8 timings reversely linked to the ooplasm diameter ($p=0.003$, $r=-0.12$, $p=0.001$ $r=-0.16$ and $p\leq0.001$ $r=-0.36$, respectively). But, there was insignificant relationship between oocyte morphometry and other morphokinetic parameters, irregular cleavage embryos as well as embryo arrest ($p>0.05$).
Conclusion: The data revealed that the oocyte morphometric parameters were not a powerful tool for prediction of embryo morphokinetic or embryo selection in ICSI cycles. However, ooplasm diameter may be useful for predicting some of the embryos cleavage timing.
Key words: *Embryo, Oocyte morphometry, Morphokinetic, Irregular cleavage.*

P-56

Is there any correlation between embryo morphokinetics development and maternal age in an intra-cytoplasmic sperm injection program?

Faramarzi A[1, 2], Khalili MA[2].
1.*Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: a.faramarzi90@gmail.com

Background: It is widely accepted that aging decreases the fertility capacity in women.
Objective: The aim of this study was to assess embryo morphokinetics parameters and cleavage pattern correlation with maternal age.
Materials and Methods: Morphokinetics of embryos derived from women <30, 30-35, 35-40 and 40≤yr were compared retrospectively in terms of time of second PB extrusion (tPB2), time of pronuclei appearance (tPNa), time of pronuclei fading (tPNf) and time of two to eight discrete cells (t2-t8). Also, abnormal cleavage patterns such as, uneven blastomeres at two cells stage, cell fusion (Fu) and Trichotomous Mitoses (TM) were assessed.
Results: The findings showed that only t5 occurred later in women 35-40 and 40≤ when compared with <30 and 30-35 yr (p=0.000). Other morphokinetics timing parameters as well as uneven blastomere were comparable (p>0.05). However, Fu and TM were increased in women with 40≤ age compared with younger women (p=0.000).
Conclusion: It is concluded that maternal age correlated to cleavage pattern of embryos. So, evaluation of embryo morphokinetics may improve embryo selection and increase fertility in advanced maternal age.
Key words: *Morphokinetics, Cleavage pattern, Maternal age, Embryo.*

P-57

Effect of GDF9 and CCs supplementation in IVM culture media on viability of blastocysts generated from vitrified cleavage embryos

Honari Chatroudi M[1], Khalili MA[2], Ashourzadeh S[3], Anbari F[2], Talebi AR[4], Shahedi A[1].
1.*Department of Anatomy and Cells Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Reproductive Biology, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Kerman Infertility Center, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran.*
4.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: khalili59@hotmail.com

Background: In vitro maturation (IVM) of immature oocytes is useful for infertile patients such as Polycystic Ovary Syndrome (PCOS). Embryo formation and Pregnancy rates in IVM oocytes are low in comparision with IVF cycles.it is essential to recognize the main factors in regulation the oocyte maturation. This can be referred to the cobe culture system and GDF-9 which is released by the oocyte.
Objective: The purpose was to investigate the effects of GDF-9 and cumulus cells (CCs) supplementation in IVM culture media on the formation and viability rates of blastocysts generated from vitrified cleavage stage embryos.
Materials and Methods: A total of 80 GV oocytes from stimulated cycles underwent the IVM program. These oocytes were divided into four groups of I) cultured in IVM media, II) IVM+ CCS, III) IVM+GDF-9 (200 ng/ml) and IV) IVM medium+ CCS+ GDF-9 (200 ng/ml). Intracytoplasmic sperm injection (ICSI) was performed for the IVM oocytes and the generated cleavage embryos were vitrified for future analysis. Following thawing, the embryos were cultured for 3 days and the developed blastocysts were assessed for viability rates with PI/ hochest immunostaining.
Results: Blastocyst formation was not significant in the three treatment groups compared to the control (group I). The mean of viability rates in the II, III and IV groups were 57.82%, 57.75% and 59.9% respectively, versus 38.78% in the control group. The rates of blastocyst viability in II, III and IV groups were significantly higher than the control group.
Conclusion: The use of GDF-9 CCs in IVM culture media enhanced the viability rate of the generated blastocysts from vitrified cleavage embryos. Therefore, modification of IVM culture media is recommended.
Key words: *IVM,GDF-9, Cumulus cells, Blastocyst formation.*

P-58

The effects of chronic administrations of Niacin on the anthropometric and ovarian morphological changes PCO rats

Izadi M[1], Asadi N[2], Aliabadi A[1], Esmaeilidehaj M[1], Rezvani ME[2], Vatankhah A[1].
1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Reproductive Physiology, School of Medical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: mizadi890112@gmail.com

Background: Polycystic ovary syndrome (PCOS) considered as a prevalent endocrine syndrome in women of reproductive age. Patients with PCOS reveal body and ovary weight alternations and substantial histological changes in the ovaries. Based on these evidences that Niacin can modulate insulin sensitivity through the adiponectin secretion, this study was conducted to evaluate the role of Niacin on PCOS complications such as body and ovary weight gain, ovarian morphological parameters in PCOS rats.
Objective: Present study was conducted to determine the effects of Niacin on different complications of PCOS.
Materials and Methods: Female Wistar rats with normal estrus cycle (n = 28) were allocated into 4 groups of seven

animals. PCOS was induced by the intramuscular injection of 4 mg estradiol valerate. The control group was treated with saline while the PCOS groups were orally administered with Niacin (25 or 50 mg/kg) and clomiphene citrate (25 mg/kg) respectively. Four weeks after the treatments anthropometric parameters were measured and ovarian morphological changes were evaluate using Hematoxtlin-Eosin staining method.

Results: The result of study revealed that Niacin at different doses reduced body weight gain and improve ovary weightin compared to control group ($p<0.05$). although the number of healthy follicles did not change in different groups, the cystic follicles were increased significantly in the PCOS and Niacin therapy could improve the increased the follicles in treated rats ($p<0.05$). Diminished corpora lutea and increase in thickness of theca interna in PCOS rats were significantly improved in rats treated with Niacin in compared to control rats ($p<0.01$).

Conclusion: Consumption of Niacin can improve body and ovary weight gain. Also, Niacin induces beneficial impacts on ovarian morphological changes that induce in PCOS.

Key words: *Polycystic ovary syndrome, Rat, Nicacin, Ovary morphology.*

P-59

The effects of Sitagliptin on the serum and ovarian inflammatory mediators of rats with polycystic ovary syndrome

Izadi M[1], Safaeian A[2], Esmaeilidehaj M[2], Aliabadi A[2], Asadi N[2], Vatankhah A[2], Rezvani M[2].

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

Email: azadeh.safaeian@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects 5-10% of women at reproductive age. Agents that improve metabolic syndrome have been useful for reducing PCOS complications. Since, the utilization of the drugs that improve metabolic syndrome and insulin resistance has clinical significance in control of PCOS and this syndrome is associated with low grade inflammatory state in the ovary, we designed this study to evaluate sitagliptin effects, as an inhibitor of DPP-4, on inflammatory biomarkers in ovary of PCOS rats.

Objective: Recently, dipeptidyl peptidase 4 (DPP-4) inhibitors that block degradation of GLP-1used to treat PCOS. We designed this study to evaluate sitagliptin effects, as an inhibitor of DPP-4, on inflammatory biomarkers changes in ovary of PCOS rats.

Materials and Methods: This research work is an interventional experimental study. After induction of the syndrome by single injection of estradiol valerate at dose of 4 mg/rat, PCO rats were divided into 3 groups including the PCOS, and the sitagliptin groups receiving daily administration of siagliptin at a dose of 25 or 50 mg/kg (P.O.) for 30 days. Thereafter, animal ovaries were removed for histological study and gene expression analysis. Blood samples were taken to evaluate serum TNF-α and CRP level. To evaluate the mRNA expression of the inflammatory mediatores (IL-6, TNF-α) after total RNA extraction. cDNA synthezied and Real time-PCR was performed. Serum levels of TNF-α and CRP were measured according to according to manufacturer kit instructions.

Results: Treatment with sitagliptin significantly reduced the mRNA expression of IL-6 and TNF-α in the ovary. Serum levels of CRP and TNF-α were reduced in sitagliptin treated rats ($p<0.05$).

Conclusion: Generally, the results of this study showed that sitagliptin can improve the conditions of polycystic ovaries syndrome. In addition, the results indicate that part of the improvement effects of sitagliptin can be mediated by reducing serum and ovarian inflammatory markers.

Key words: *PCOS, Sitagliptin, Inflammatory, Rat.*

P-60

The effect of cytoplasm microinjection in embryo development and expression of HOXA10 in mouse

Kiani Feyzabadi M[1], Salehi M[2], Mogheiseh A[1].

1. *Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.*
2. *Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: maryamkiani175@yahoo.com

Background: One of the main technologies for the genetic investigations is creating transgenic mice. This goal has recently been achieved by pronuclear microinjection of plasmid DNA into fertilized egg. The survival of the embryo and the success rate in this method is very low.

Objective: Therefore, the aim of this study was to investigate the effect of cytoplasm microinjection method in embryonic development and expression of HOXA10 as an effective gene in fertility and embryo viability.

Materials and Methods: Female mice were superovulated with pregnant mare serum gonadotropin followed by human chorionic gonadotropin. For IVF, 15-16h after hCG injection, cumulus oocyte complexes were poised in improved HTF medium. Spermatozoa were collected from the caudal epididymis. Cumulus-oocyte complexes were inseminated with capacitated spermatozoa in a humidified atmosphere of 5% CO_2, 95% air at 37^{o}C. Six hours after insemination, the fertilized oocytes cultured. IVF embryos, zygotes, divided into two groups: the first group was control and the second was plasmid (Without any gene) injection in cytoplasm. After 24 and 96 hr of culture, the number of two cell and expand blastocysts rates were recorded. Expression of HOXA10 assessment by quantitative realtime PCR (qPCR blastocyst stage.

Results: The results obtained from the analysis of embryo development, a slight decrease was observed in

the percentage of fertilization and blastocyst in the cytoplasm microinjection compared to the control group (82.38±5.5, 53.77±6.9 vs. 96.19±1.03, 61.50±5.08 control) but this decrease was not significantly different. Degeneration rate in cytoplasm micro injection was significantly higher than the control group (42.59±5.5 vs. 6.40±2.6). Real Time PCR results showed the amount of HOXA10 gene expression in cytoplasm microinjection group has decreased relative to control.

Conclusion: Considering the simplicity of this method in comparison to the pronuclear microinjection and based on the results, which showed that this method has a good efficiency, the small difference in results with the control group can be due to mechanical manipulation, but it can be suggested that this method is a good alternative to pronuclear microinjection.

Key words: *Microinjection, HOXA10, Mouse, Embryo development.*

P-61

Is there any correlation between sperm parameters and chromatin quality with embryo morphokinetics in patients with male infertility?

Mangoli E[1], Khalili MA[1], Talebi AR[1], Ghasemi-Esmailabad S[2], Hosseini A[1].

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

2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: khalili59@hotmail.com

Background: Human sperm DNA damage may adversely affect the embryo quality.

Objective: The main goal was to evaluate the correlation between sperm parameters and chromatin quality with embryo kinetics via time-lapse monitoring system (TLM) in a single embryo transfer (SET).

Materials and Methods: A total of 40 couples involved in the ICSI program as a result of male infertility. For assessment of sperm chromatin and DNA quality, we used aniline blue, toluidine blue, chromomycin A3, acridine orange and terminal transferase-mediated deoxyuridine triphosphate biotin end labelling assays. All mature oocytes were injected, and the generated zygotes (2PNs) were cultured in TLM. In day 3 after injection, single embryo transfer (SET) was carried out according to the morphology and morphokinetics. The patients were followed up until delivery.

Results: There were positive significant correlations between sperm count with CC2 (r=0.330, p=0.049), T4 (r=0.329, p=0.038), T6 (r=0.342, p=0.035) and T7 (r=0.374, p=0.025). Also, there were positive significant correlations between non-progressive motility and T2 (r=0.323, p=0.042), T3 (r=0.411, p=0.013) and T4 (r=0.418, p=0.007). Regarding the sperm chromatin quality assays, there were negative significant correlations between CMA3 and CC2 (r=0.272, p=0.049) and between acridine orange and T5 (r=0.221, p=.040). There were also significant differences between t2 with implantation rate (p=0.039) and t3 with clinical pregnancy rate (p=0.032). None of the tests had significant differences with ART outcomes except TB test that had noticeable relationship with the LBR (p=0.035).

Conclusion: It seems that the abnormal sperm parameters and chromatin alteration had influential roles on embryo kinetics in patients with male factor infertility.

Key words: *Chromatin, Embryo kinetics, Intracytoplasmic sperm injection, Sperm parameters, Time lapse.*

P-62

Effect of estradiol on oocyte qualitative and quantitative parameters and embryo formation following IVF in mice

Mohammadzadeh M[1, 2], Anbari F[1], Motamedzadeh L[1], Akhavansales Zh[3], Ghasemi-Esmailabad S[1], Shamsi F[4], Khalili MA[1].

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

2.*Medical Nanotechnology & Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

3.*Department of Immunology, International Campus, Shahid Sadoughi Universirt of Medical Sciences, Yazd, Iran.*

4.*Department of Biostatistics and Epidemiology, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: khalili59@hotmail.com

Background: oocyte maturation is one of the important role in vitro fertilization. aging affects on the genome activation which is related with oocyte maturation. advanced age cause changes in oocyte inclusive cytoplasmic/ extracytoplasmic deficiencies. Studies showed use of exogenous estrogen and antioxidants can regulate apoptosis process and reduce oocyte cytoplasm abnormalies.

Objective: Therefore, our aim in this study was improve the oocyte quality parameters by using appropriate doses of estradiol and sesame oil.

Materials and Methods: Six groups of mice were selected, 3 groups young and 3 groups old. the control group received normal saline and exprimental group received sesame oil (10 IU) and estradiol (E_2) 1 µl + sesame oil (10 IU) Respectively., all treatments was done intraperitoneally in diestrus phase. after IVF program the status of Refractile body, smooth endoplasmic reticulum, vacuole, oocyte cytoplasm polar body, previtelline space, zona pellucida thichness, oocyte number and two cells embryo were evaluated.

Results: in young group, oocyte number was higher than old ones (p=0.015) But the results are beneficial with the group who received sesame oil (29.70±2.43 vs 23.10±2.43 p=0.01). The pervitiline space in the young group that received (E_2) + sesame oiLwas less than the old one (p=0.015). The number of two-cell embryos in sesame oiL group was higher than other groups (p=0.009). there was no significant difference in the

appearance of cytoplasm,pollar body, SER, Vacuole and Perivitelline space except the number of refract body in old group (p=0.016).

Conclusion: This study demonstrate that the sesame oil as a strong antioxidant will have beneficial effects on the number of oocytes and two cell embryo in young group. the result shows that advance age dose no change in oocyte quantitative parameters except refractile body.

Key words: *Estradiol, Sesame oil, Oocyte Morphology, Embryo formation, IVF, mice.*

P-63

Evaluation of maternal diet effects on body and ovary of female mouse offsprings

Mollahasan N[1], Alizadeh AR[2], Ebrahimi B[2], Ghasemi ZS[2], Fatehi R[2], Akbarinehad V[3].

1.*Department of Biology, Faculty of Sciences, University of Science and Culture, Tehran, Iran.*
2.*Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3.*Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*

Email: Alizadehmasouleh@royaninstitute.org

Background: Although polyunsaturated fatty acid (PUFA) and antioxidants are essential nutrients known to be required for reproduction, an increasing trend was observed in the consumption of n-6 fatty acids (FA) rather than n-3 FA. Besides, the key roles of omega-3 FA in maternal nutrition on offspring reproduction has not been studied yet.

Objective: Our main objective was to investigate the effects of a maternal diet supplemented with fish oil and vitamin E on offspring's body and ovarian weight.

Materials and Methods: Forty female mice were randomly divided into: Control (Cont), Fish Oil (FO), Vitamin E (VE) and Vitamin E + Fish Oil (VEFO) diet. Mother mice were fed experimental diets from 14 days before mating till the end of the breastfeeding period. After weaning, offspring were fed standard diet and evaluated at puberty, 7 months and 10 months of age.

Results: At puberty (9 wk old mice), body weight was significantly higher in VEFO group than Cont and VE. In 7 and 10 months old mice, body weight in FO and VEFO was significantly higher than VE group. Ovary weight in 7 and 10 months old mice was higher than 9 wk old mice ($p<0.0001$), it was approximately similar in Cont and VEFO groups and was higher than VE group ($p<0.01$).

Conclusion: Although vitamin E supplementation to maternal diet alone showed negative effects on body and ovary weight, combination of vitamin E with fish oil in maternal diet increase body and ovary weight in 7 and 10 months old female offspring. Of course, it warrants further studies.

Key words: *Vitamin E, Fish oil, Maternal diet, Offspring ovary.*

P-64

Aberrant expression of Igf2 imprinted gene in mouse blastocysts following vitrification at 2-cell stage

Movahed E[1], Salehi M[2], Akbari Sene A[3].

1.*Department of Anatomy, Faculty of Medicine Iran University of Medical Sciences, Tehran, Iran.*
2.*Department of Biotechnology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3.*Shahid Akbar-Abadi Hospital IVF Center, IVF Department, Iran University of Medical Sciences, Tehran, Iran.*

Email: elhammovahed92@gmail.com

Background: Embryo vitrification is an important approach in assisted reproductive techniques (ART) clinics. It helps to prevent ovarian hyperstimulation syndrome and multiple gestations by preserving excess embryos. However, few studies have investigated the effects of vitrification on genomic imprinting of preimplantation embryo.

Objective: Thus, this study aimed to assess the effect of vitrification on Igf2 expression as an important imprinted gene in preimplantation embryo.

Materials and Methods: Expression of Igf2 in mouse blastocysts from control, IVF and vitrification groups was investigated by qRT-PCR.

Results: The results showed that quantitative level of Igf2 gene was significantly lower in blastocysts derived from vitrification in comparison to control and IVF blastocysts ($p<0.05$).

Conclusion: In conclusion, vitrification causes disruption in genomic imprinting mechanism in blastocysts.

Key words*: IVF, Genomic imprinting, Igf2, Blastocyst.*

P-65

Reporting experience on more than 100 blastomeres for diagnosing X-linked disorders

Sabeghi S, Keshvar Y, Fatemi K, Younesi S, Fouladi P, Sharifi Z, Bandehi Sarhadi A, Shirzad T, Bagherian H, Zeinali S.

Dr. Zeinali's Medical Genetics Lab, Tehran, Iran.

Email: zeinali@gmail.com

Background: Preimplantation genetic diagnosis (PGD) is a useful option for couples with a risk of transmitting a genetic disease to prevent birth of children affected with monogenic disorders. In this method, diagnoses performed on blastomeres biopsied from 8-cell stage embryos which are created by in vitro fertilization method (IVF). In this way, one could prevent medical abortion by selecting and transferring unaffected embryos. Other usages of PGD are sex selection and HLA typing. Also PGD allows infertile couples who are potentially candidate for IVF and just have one chance to get pregnant, assure their child's sex or health before pregnancy.

Objective: Here we present application of molecular PGD to select unaffected embryos for couples who are carrier of X-linked disorders such as hemophilia A, hemophilia B and Duchenne muscular dystrophy (DMD).

Materials and Methods: 19 carrier couples who were candidate for PGD referred to our laboratory. Peripheral blood samples were collected and genomic DNA was extracted using salting out method. Mutation detection carried out using Sanger sequencing method. Fragment

analysis and haplotype mapping performed to trace defective alleles in the family using multiplex short tandem repeats (STRs). On day 3 post fertilization a blastomere removed from each embryo. Selected mutation and informative STR markers were checked for each blastomere using nested PCR method. Linkage analysis performed and intended embryos were selected and implanted to mother's uterus.

Results: Since 2009, 140 blastomeres were checked for X-linked disorders. 89 of them were for diagnosing hemophilia A, 16 for hemophilia B and 35 for DMD. From this amount 60 embryos were checked for aneuploidy abnormalities (QF-PCR) in parallel. Totally 81 embryos were transferable which resulted in pregnancy and child birth in 8 cases.

Conclusion: PGD is regarded as a powerful diagnostic tool for carrier couples who desire a healthy child and wish to avoid medical abortion. In case of having limitation for performing sequencing in some cases which a large deletion causes the disorder, which usually happens in diseases such as DMD, results obtained from linkage analysis and haplotype mapping are enough reliable to detect unaffected embryos.

Key words: *PGD, IVF, STR, X-linked disorders.*

P-66

Vitamin D3 regulates mtDNA copy number, mitochondrial biogenesis and membrane integrity in granulosa cells through mitogen activated protein kinase-extracellular signal-regulated kinase (MAPK-ERK1/2) pathway in a mouse model of polycystic ovary syndrome

Safaei Z[1], Shirazi R[1], Bakhshalizadeh S[2], Akbari Sene A[3], Nasr-Esfahani MH[4], Soleimani M[1].

1. *Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2. *Department of Anatomical Sciences, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.*
3. *Department of Obstetrics and Gynecology, Shahid Akbarabadi Hospital IVF Center, Iran University of Medical Sciences, Tehran, Iran.*
4. *Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.*

Email: Zahrasafaie94@gmail.com

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women. Menstrual dysfunction, anovulation, hyperandrogenism, hirsutism and polycystic ovaries are regarded as signs and symptoms of PCOS. Hyperandrogenism intensify follicular atresia by apoptosis in granulosa cells. So, apoptosis and oxidative stress can disturb follicular growth in PCOS women. Since apoptosis are regulated by mitochondria, these organelles may be affected in PCOS by high rate of follicular atresia. Therefore, granulosa mitochondrial function disturbance causes some problems in oocyte function and might decrease fertility and pregnancy rate. The aim of study was investigation the effect of vitamin D3 on mtDNA copy number, mitochondrial biogenesis and membrane integrity via the MAPK pathway in granulosa cells of PCOS mouse model.

Objective: The effect of vitamin D3 on mtDNA copy number, mitochondrial biogenesis and membrane integrityin granulosa cells derived from mouse model of poly cystic ovarian syndrome (PCOS) via mitogen-activated protein kinase (MAPK-ERK1/2).

Materials and Methods: In this study, the PCOS model was triggered by injection of dehydroepiandrosterone (DHEA). Then, isolated granulosa cells were treated with vitamin D3, MAPK activator and inhibitor. Granulosa cells isolated from PCOS ovaries after identification by FSHR (granulosa cell marker) and CD45 (leuckocyte marker) were cultured in six groups: (1) granulosa cells treated with vitamin D3 (100 nM for 24 hr); (2) granulosa cells treated with MAPK activator (10 µM for 4 hr); (3) granulosa cells treated with MAPK inhibitor (10 µM for 4 hr); (4) granulosa cells treated with vitamin D3 and MAPK inhibitor; (5) granulosa cells treated with MAPK inhibitor and MAPK activator; (6) non-treated granulosa cells (control group). Mitochondrial biogenesis gene expression was compared between different groups using real-time PCR. mtDNA copy number was investigated by qRT-PCR. Mitochondrial membrane integrity was evaluated by transmission electrone microscopy (TEM).

Results: Mitochondrial biogenesis (TFAM, NRF2) were downregulated in granulosa cells of PCOS mice when compared to normal mice, but treatment with vitamin D3 and MAPK activator increased mRNA expression levels of these genes. To evaluate the alterations of mitochondrial structure, transmission electron microscopy was used. Most of the mitochondria in PCOS group without any treatments and in PCOS group that treated with vitamin D3 and MAPK inhibitor and also the group that treated with MAPK activator and MAPK inhibitor were spherical with almost no cristae. Our results showed that in PCOS group that treated with vitamin D3 and MAPK activator, the mtDNA copy number increased significantly in comparison to control group (non-treated PCOS group) but in PCOS group that treated with vitamin D3 and MAPK inhibitor and also the group that treated with MAPK activator and MAPK inhibitor the mtDNA copy number decreased.

Conclusion: This study suggests that vitamin D3 improve mtDNA copy number, mitochondrial biogenesis and membrane integrity through MAPK pathway in granulosa cells of PCOS mice that may improve follicular development and oocyte quality.

Key words: *MAPK-ERK1/2 pathway, Granulosa cell, Polycystic ovary syndrome, Mitochondrial biogenesis, mtDNA, Mitochondrial membrane integrity, Vitamin D3.*

P-67

L-Carnitine reduces the adverse effects of ROS and up-regulates the expression of implantation related genes in in vitro developed mouse embryos

Shafiei G, Almasi M, Nikzad H, Moshkdanian G.

Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: golnazshafie99@gmail.com

Background: In vitro developed embryos are inevitably exposed to various reactive oxygen species (ROS) which may decrease the embryos competence for ART purpose. Optimization of embryo culture media using antioxidant agents could help to improve the embryo quality and could overcome the mentioned issue.
Objective: The aim of this study was to evaluate the effects of L-carnitine (LC) in culture media on early embryo competence and expression of ErbB1 and ErbB4 implantation related genes.
Materials and Methods: Two-cell mouse embryos were cultured in four following conditions: LC group which transferred into a media containing L-carnitine; H_2O_2 group which exposed to H_2O_2 for 30 min and then transferred into a simple media; H_2O_2+LC group which exposed to H_2O_2 for 30 min and then transferred into a media containing L-carnitine; the simple group which only kept throughout in simple media. All groups were allowed to develop until the blastocyst stage. Then the ErbB1 and ErbB4 expression were evaluated by Real-time PCR and immunocytochemistry. The expression of Sirt3 gene was also evaluated. Moreover, intracellular ROS level was examined by DCFH-DA fluorescence intensity. In order to assess the morphological quality of the embryos, ICM and OCM number blastocyst cells were evaluated by using Hoechst and propidium iodide (PI) staining. The ErbB1, ErbB4, ROS levels and the number of cells were also compared with an in vivo developed blastocysts model.
Results: Our data revealed that L-carnitine significantly increases the ErbB1 and ErbB4 gene and protein expression as implantation related genes. But, intracellular ROS level and consequently Sirt3 gene expression were significantly decreased after L-carnitine treatment. It is worth noting that an elevated cell number was observed in the LC-treated group compared with the other groups.
Conclusion: Our findings suggest that the use of L-carnitine could be a helpful factor to improve the preimplantation embryo culture media through the decrease of ROS level and the increase of implantation-related genes.
Key words: *L-carnitine, Embryonic Implantation genes, Gene expression, ROS.*

P-68

The effect of encapsulating the ovaries with alginate hydrogels on the expression of caspase3 gene in the vitrified ovaries of mice

Shirazi Tehrani A[1], Mazoochi T[2].
1. *Student Research Committee, Kashan University of Medical Sciences, Kashan, Iran.*
2. *Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
Email: mazoochi45@yahoo.com

Background: Cryopreservation of the ovarian tissue is one of the methods for preserving fertility, which, unlike cryopreservation of the oocyte and embryo, can be done before puberty and at each stage of the menstrual cycle. Apoptosis may be induced by the process of cryopreservation due to changes in physical conditions. When induced by apoptosis in the cell, the cell death cascade activates the caspases and, as a results of cell damage, can affect the quality of the ovarian reserve; Encapsulation is a method that has been investigated and proposed to protect tissues and cells in different conditions.
Objective: Therefore, due to the importance of apoptosis after vitrification and warming, the aim of this study was to determine the effect of hydrogel alginate as capsule on the expression of caspase 3 gene in the vitrified mouse ovaries.
Materials and Methods: In this experimental study, the ovaries of adult mice were divided into 4 groups: non-vitrified ovaries, vitrified ovaries, ovaries encapsulated in alginate hydrogel at 0.5 and 1% concentration and then vitrified (respectively, Experimental group 1 and 2). Vitrification was performed with ethylene glycol and dimethyl sulfoxide solutions. The duration of exposure to liquid nitrogen was 30 minutes and warming the ovary with decreasing sucrose solutions. The level of expression of caspase3 gene was evaluated in each group with real time RT-PCR. The HPRT gene was considered as the reference gene. Data were analyzed by one-way ANOVA and post hoc tests. The values of $p<0.05$ were considered statistically significant.
Results: Molecular studies showed that caspase3 gene was expressed in all groups. Non-vitrified group was considered as 1 and the other groups were compared to it. The expression level of this gene was higher in the experimental groups than in the vitrified group, but no significant difference was found between the experimental and the vitrified group. The lowest expression of this gene was observed in the experimental group 2 (1% concentration).
Conclusion: Encapsulation of the ovaries with concentrations of 0.5 and 1% of alginate hydrogels did not reduce the expression of caspase3 gene in vitrified ovaries.
Key words: *Vitrification, Alginate hydrogel, Apoptosis, Ovary, Mice.*

P-69

Zona pellucida birefringence and meiotic spindle visualization are not related to the time-lapse detected embryo morphokinetics in women with polycystic ovarian syndrome

Tabibnejad N, Soleimani M, Aflatoonian A.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: abbas_aflatoonian@yahoo.com

Background: Oocyte parameters as well as embryo developmental kinetics can be applied as non-invasive predictors for selection of viable embryos and clinical outcomes.

Objective: Time-lapse morphokinetic events in conjunction with zona pellucida birefringence (ZPB) and meiotic spindle visualization (MSV) have been evaluated for predicting pregnancy outcome.
Materials and Methods: We assessed a total of 547 embryos from 100 intra cytoplasmic sperm injection cycles in women with polycystic ovary syndrome (PCOS) and tubal factor infertility. ZPB and MSV were evaluated in 100 oocytes using polarized microscope. Time-lapse archives were noted for time to pronuclear fading (tPNf), time to 2-8 cells (t2-t8), direct cleavage, reverse cleavage and also for the presence of multinucleation.
Results: The mean timings of embryo morphokinetics were not significantly different between high and low ZPB and visible and not-visible meiotic spindle oocytes in the PCOS group ($p>0.05$). Furthermore, implantation rate was 4.6 fold higher in embryos derived from oocytes with visible meiotic spindles compared to those without visible meiotic spindle in PCOS patients ($p<0.05$). We observed that chemical and clinical pregnancy rates as well as live birth were higher in transferred embryos which formed from oocytes with visible meiotic spindle (27.6% vs.16.7%), (24.1% vs. 8.3%) and (24.1% vs. 8.3%) respectively. According to logistic regression analysis, only tPNf significantly influenced clinical pregnancy and live birth ($p<0.05$).
Conclusion: Time-lapse embryo parameters are not related to oocyte ZPB and MSV status. However, clinical pregnancy and live birth have been affected by the presence of meiotic spindle.
Key words: *Meiotic spindle visualization, PCOS, Pregnancy outcome, Time-lapse embryo morphokinetic, Zona pellucida birefringence.*

P-70

Assessing ICSI outcome by combining non-invasive indicators: Early time-lapse morphokinetics and apoptosis in associated cumulus cells among women with the polycystic ovarian syndrome

Tabibnejad N[1], Aflatoonian A[2], Motamedzadeh L[1], Soleimani M[1], Sadeghian-Nodoushan F[3], Talebi AR[2].
1.*Department of Reproductive Biology, Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Advanced Medical Sciences and Technologies, Medical Nanotechnology and Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: prof_talebi@hotmail.com

Background: Cumulus cells features and embryo developmental events can be considered as noninvasive indicators for embryo selection and clinical outcomes.
Objective: A combination of time-lapse morphokinetic parameters and cumulus cell apoptosis in women with polycystic ovarian syndrome (PCOS) was evaluated for predicting pregnancy outcome.
Materials and Methods: We assessed a total of 547 embryos from 100 intracytoplasmic sperm injection (ICSI) cycles. Time-lapse records were interpreted in time to pronuclear fading (tPNf), time to 2 to 8 cells (t2-t8), direct cleavage, reverse cleavage, and also for the presence of multinucleation. Percentages of apoptosis were identified in 100 associated cumulus cell samples using the TDT-mediated dUTP-biotin nick end-labeling assay.
Results: The significant decrease of apoptotic cumulus cells was detected in patients with chemical and clinical pregnancies as well as live birth among patients PCOS and in the tubal infertility group ($p>0.05$). Furthermore, significantly higher implantation rate and also significantly lower cases of early pregnancy loss were observed in the group of oocytes with less apoptotic cumulus cells. Multivariate logistic regression analysis showed that tPNf together with cumulus cell apoptosis were independent prognostic factors of chemical pregnancy, clinical pregnancy rate, and live birth.
Conclusion: Time-lapse embryo parameters may not reflect the cumulus cell apoptosis rate. However, the rate of apoptotic cumulus cells is significantly associated with ICSI outcome using Day 3 embryo transfer.
Key words: *Apoptosis, Cumulus cell, PCOS, Pregnancy outcome.*

P-71

Serum anti-Mullerian hormone and embryo morphokinetics detecting by time-lapse imaging: A comparison between the polycystic ovarian syndrome and tubal factor infertility

Tabibnejad N, Soleimani M, Aflatoonian A.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: abbas_aflatoonian@yahoo.com

Background: Anti-Mullerian hormone (AMH) is considered as a good marker for quantitative evaluation of ovarian response to the stimulation during assisted reproductive technology cycles.
Objective: To evaluate the association between serum AMH level and embryo morphokinetics using time-lapse imaging and intracytoplasmic sperm injection (ICSI) outcomes in women with polycystic ovarian syndrome (PCOS).
Materials and Methods: We evaluated a total of 547 embryos from 100 women underwent ICSI cycles; 50 women with PCOS and 50 women with tubal factor infertility. Serum AMH level was measured in all participants. Time-laps records were annotated for time to pronuclear fading (tPNf), time to 2-8 cells (t2-t8), reverse cleavage, direct cleavage, and also for the presence of multinucleation.
Results: AMH was negatively correlated with t5, t8, and the third cell cycle ($p=0.02$, $p=0.02$, and $p=0.01$; respectively) in PCOS group. AMH had no correlation with embryo kinetics in infertile women with tubal factor infertility. Moreover, AMH level is similar between

embryos with and without direct cleavage as well as reverse cleavage and Multinucleation in both groups. The Receiver operating characteristic curves analyses indicated that AMH was not an accurate predictor of clinical pregnancy as well as a live birth (AUC=0.59 [95% CI, 0.42-0.76]) in PCOS women. However, in the women with tubal factor infertility AMH showed a fair prediction value for clinical pregnancy (AUC=0.64 [95% CI, 0.48-0.82]) along with the live birth (AUC=0.70 [95% CI, 0.55-0.85]).

Conclusion: Some of the time-lapse embryo parameters may be related to the AMH concentration. However, AMH is not an accurate tool to predict the ICSI outcomes in PCOS women.

Key words: *Anti-Mullerian hormone, Embryo morphokinetic, PCOS, Pregnancy outcome, Time-lapse.*

P-72

Fluctuations between estradiol level and cumulus oocyte complex (COC) count: Does it predict the outcomes of ART cycles?

Taheri F[1], Omidi M[1], Khalili MA[1], Agha-Rahimi A[1], Sabour M[1], Framarzi A[2], Mangoli E[1].

1.*Department of Reproductive Biology, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

2.*Department of Anatomical Sciences and Biology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

Email: 63rahimi@gmail.com

Background: The success of in vitro fertilization (IVF) depends on controlled ovarian hyperstimulation (COH) resulting in multi-follicular response. The follicles contain granulosa cells which secrete hormone estradiol (E2). E2 is required for follicular development and play an important role in embryo implantation.

Objective: The aim was to assess the impact of total serum E2 on the day of human chronic gonadotropin (hCG) administration and the serum E2 per oocyte ratio in the outcomes of assisted reproductive technology (ART) cycles.

Materials and Methods: A total of 205 women were categorized into 3 groups according to the serum E2 levels: 1: <1500 pg/ml; 2: 1500-3000 pg/ml; 3: >3000 pg/ml. Another categorization includes 3 groups according to E2/oocyte ratio: A: <150 pg/ml per oocyte; B: 150-200 pg/ml per oocyte; and C: >200 pg/ml per oocyte. The outcome compared between groups include laboratory and clinical characteristics. One-way analysis of variance (ANOVA), Chi-square and Kruskal-Wallis tests were performed wherever appropriate. differences were considered significant at $p<0.05$.

Results: There was a significant difference between the groups based on the E2 levels with respect to laboratory parameters. In group 3, the rates of chemical pregnancy (50%), clinical pregnancy (54.1%) and live birth (45.8%) were significantly higher when compared to the other groups ($p=0.004$, $p<0.0001$; $p<0.0001$, respectively). Moreover, according to E2/oocyte ratio, the chemical pregnancy rate was higher in group A (46.6%, $p=0.002$); and the clinical pregnancy (40.5%) and delivery (29.7%) rates were higher in group C ($p<0.001$).

Conclusion: Supraphysiological levels of E2 resulting had no adverse effects on the quality of the embryos in IVF cycles. Therefore, the E2/oocyte ratio data can be a useful adjunct in predicting success rates of IVF cycles. It is confirmed that both pregnancy and live birth rates were elevated with E2/oocyte ratio >200 pg/mL.

Key words: *E2, oocyte ratio, Estradiol level, IVF, Live birth rate.*

P-73

Generation of viable blastocysts from discarded human cleavage embryos

Taheri F[1], Khalili MA[1], Kalantar SM[2], Fesahat F[3], Montazeri F[2], Palmerini MG[4], Woodward B[5].

1.*Department of Reproductive Biology, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

3.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

4.*Department of Health Sciences, Faculty of Medicine, University of L"Aquila, Italy.*

5.*X &Y Fertility, Leicester, UK.*

Email: Khalili59@hotmail.com

Background: By improving the culture systems, demonstrated that some discarded poor quality embryos can reach to blastocyst and are capable to implantation.

Objective: This study investigated blastocyst developmental competence and euploidy status in human embryos that had been classed as too poor quality to transfer (ET) or cryopreserve at the cleavage stage.

Materials and Methods: Embryos were divided into three groups. Group 1 (n=41) included good quality embryos from candidates of preimplantation genetic testing for aneuploidy (PGT-A). Groups II and III were the 'rejected' supernumerary embryos, defined as suboptimal for ET or vitrification after morphological examination, with embryos randomly divided between the groups. Group II embryos (n=31) were cultured up to the Day 3 cleavage stage, when they were biopsied and fixed. Group III embryos (n=27) were cultured up to the Day 5 blastocyst stage, when they were evaluated for morphology and chromosomal status. Chromosomal status in all groups was assessed by multi–color fluorescence in-situ hybridization (FISH) for chromosomes 13, 18, 21, X and Y.

Results: Euploidy rates in Groups I, II and III were 56.1%, 38.7%, and 55.5%, respectively. Among the blastocysts that developed from 'rejected' embryos, 59.3% were classed as good quality. The most frequent chromosomal aneuploidy was related to the sex chromosome (22.2%). The mosaicism rate was not significantly different between the Group II and III embryos (25.8% vs. 37.0%, $p=0.28$). The distribution of sex chromosomal aneuploidies was significantly different

in Group III embryos (22.2% male vs. 55.5% female, p<0.0001).
Conclusion: In conclusion, surplus poor-quality embryos rejected from clinical utilization at the cleavage stage may develop into viable blastocysts with normal chromosomal status for at least 5 chromosomes. Recovery of euploidy during poor-quality embryo transition from cleavage stage to blastocyst, could provide an alternative choice for ET.
Key words: *Blastocyst, Poor quality embryo, FISH, Aneuploidy.*

P-74
Fertilization failure following ICSI

Tanhaye Kalate Sabz F[1], Ashrafi M[2], Amjadi FS[1], Zandieh Z[1], Bashiri Z[1].
1.*Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: fateme.1694@yahoo.com

Background: Male infertility can be resolved considerably by the intracytoplasmic sperm injection (ICSI), but the total fertilization failure (TFF) following this method occurs for about 3% of cases. It is very valuable to discover the causes of this complication and find a way to solve this problem.
Objective: To discover the causes of fertilization failure following ICSI and find a way to solve this problem.
Materials and Methods: To access related articles, PubMed, Science Direct and Google Scholar were used.
Results: Total fertilization failure following ICSI may occur due to the defects in the oocyte, spermatozoa and can be due to technical problems. Defects in the activation of oocytes seem to be the most important reason, other factors include poor oocyte morphology, defects in M II oocyte spindle and sperm aster formation and premature sperm chromatin condensation. Treatment with artificial oocyte activation (AOA) may improve ICSI outcomes. There are several methods for AOA, including chemical, Physical and Mechanical activation. Chemical activation include calcium ionophores, such as ionomycin, Calcimycin (A23187) and puromycin increase membrane permeability to extracellular Ca2+. Physical activation (electro stimulation) forms pores in the plasma membrane. Mechanical activation can be acquired by aspiration of the oocyte cytoplasm may increase the oocyte calcium load at the time of injection. Sperm-related factors (morphology, motility, DNA integrity, viability) play important role in ICSI outcome. Morphologically selected sperm injection (IMSI), physiological ICSI (PICSI) technique for sperm selection can be used for patients with a history of TFF to reduce this complication.
Conclusion: Fertilization failure following ICSI is a real challenge. The factors cause TFF should be carefully investigated and well documented to minimize the risk of recurrent TFF, and it seems IMSI combined by AOA is suitable to avoid TFF.
Key words: *Fertilization failure, ICSI, Artificial oocyte activation, Sperm.*

P-75
Effective dosage of GDF9 in folliculogenesis and angiogenesis in the sheep ovarian tissues grafted onto chick embryo chorioallantoic membrane

Vatanparast M, Moshrefi M, Khalili MA.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran,
Email: khalili59@hotmail.com

Background: Many researchers have tried to culture and transplant the ovarian tissues, to preserve fertility in cancer patients. However, one of the main limitation to the applicability of this technique is the folliculogenesis disruption after transplantation.
Objective: Due to the roles exerted by Growth Differentiation Factor-9 (GDF-9), we decided to determine the most effective dose of GDF9 on promotion of folliculogenesis and angiogenesis in sheep OTs grafted onto the Chick chorioallantoic membrane (CAM).
Materials and Methods: Fresh sheep ovarian tissues were grafted onto the CAM for 5 days, and divided into four groups based on the addition of increasing doses of GDF9 (0, 150, 200, and 250 ng/ml). Following 5 days of culture, the tissues were retrieved and histological (H&E staining) and immunohistological studies (with Ki-67) were done. Fibrotic and necrotic surface were measured using MICROVISIBLE software. For comparing follicle percentage between groups, as well as differences in Ki67-positive follicles, ANOVA was applied.
Results: In the 200 and 250 ng/ml GDF9 groups, significantly higher rates of intermediary and primary follicles were observed, accompanied by a decrease in proportion of primordial follicles. The numbers of good quality follicles increased in aforementioned groups. In respect to the 0 (control) and 150 ng/ml GDF9 groups, the presence of fibrotic and necrotic areas decreased. Moreover, in the 200 and 250 ng/ml GDF9 groups, the number of capillaries and the proliferative activity increased. These results showed that higher dosages of GDF9 (200 and 250 ng/ml) were able to improve follicular development, Transplantation features, in ovarian tissue grafted onto the CAM due to improvement in angiogenesis.
Conclusion: Folliculogenesis didn't take place in ovarian grafted onto the CAM. addition of high dosages of GDF9 to the OTs, grafted onto the CAM, activated folliculogenesis in vitro, leading to higher rates of growing follicles, better blood supply, and granulosa cells proliferation.
Key words: *GDF9, Folliculogenesis, Ovary, Chorioallantoic membrane.*

3- Genetic

P-76
Evaluation of the association between promoter polymorphism of *Fas* gene with POF

Akhavansales Zh[1], Ashrafzadeh HR[2], Bitaraf Sani M[3], Montazeri F[2], Tahoori M[1], Mohammadzadeh M[4], Mirabutalebi SHR[5], Mohammadi M[4], Ghasemi N[2].

1.*Department of Immunology, Faculty of Medicine Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Animal Science Research, Yazd Agriculture and Natural Resources Research and Education Center, Yazd, Iran.*
4.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5.*Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: ghasemi@ssu.ac.ir

Background: Premature ovarian failure (POF) is the term usually used to describe women aged younger than 40 years, who identify with hypergonadotropic hypogonadism, amenorrhoea, and infertility. The clinical presentation is diverse, and several disorders can lead to POF. Alteration of the FAS pathway regulating cell death may lead to POF, but the effects of functional promoter polymorphisms of the *FAS* gene on risk of POF are unknown.
Objective: The aim of present study is the evaluation of *FAS* polymorphisms on the risk of POF patients.
Materials and Methods: We genotyped the *FAS* _670 A>G polymorphism in 51 case patients with POF and 60 control subjects by using PCR-RFLP method.
Results: Regarding the *FAS* _670 the frequency of AG genotype was 47.1% and 46.7% in POF patients and control group, respectively. The AG genotype was more prevalent in the cases (47.1%) than the controls (46.7%). Also the most POF patients had AG genotype when compared to *FAS* _670 AA and GG genotypes. However the difference was not statistically significant (OR= 1.00, p=0.99).
Conclusion: Our finding showed that the incidence of different genotype and allele frequencies of *FAS* _670 A/G was not statistically significant among POF patients and healthy women.
Key words: *Premature ovarian failure, FAS, Polymorphism.*

P-77
Identification of circular RNA hsa_circ_0060927 and CYP24A1 ectopic expression in uterine leiomyomas

Fazeli E[1], Piltan S[1], Sadeghi H[1], Gholami M[2], Yassaee F[3], Mirfakhraie R[1, 4].

1.*Department of Medical Genetics, School of Medicine, Shahid Beheshti Univarsity of Medical Sciences, Tehran, Iran.*
2.*Department of Biochemistry and Genetics, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran.*
3.*Department of Obstetrics and Gynecology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
4.*Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Email: reza_mirfakhraie@yahoo.com

Background: Uterine Leiomyomas (ULMs) are monoclonal, benign tumors that arise from the smooth muscle cells of the uterus. Regardless of their benign nature, ULMs may cause significant reproductive and gynecological complications during the reproductive years. Despite increasing researches, the molecular mechanisms underlying ULMs tumorigenesis and development are not fully understood; however Recent studies suggest that hypovitaminosis D plays an important role in uterine leiomyomas pathology. CYP24A1 encodes a mitochondrial enzyme that catalyzes the degradation of 1,25(OH)2D3, the active form of vitamin D, controlling the amount of active vitamin D in serum and many human tissues. Overexpression of CYP24A1 is reported in several human tumors and it is associated with cancer development, progression, and poorer prognosis. Circular RNAs (circRNAs) are a novel class of non-coding RNAs that their role in tumor biogenesis and progression has been proved in a number of human tumors; however, up to now, the relation between circRNAs and uterine leiomyomas remains unclear. Hsa_circ_ 0060927 is a circRNA and is derived from CYP24A1 gene, containing its exons 3-11.
Objective: In the present study, we have evaluated the expression levels of hsa_circ_0060927 and CYP24A1 in uterine leiomyoma and adjacent tissues considering the MED12 mutation profile.
Materials and Methods: The expression levels of hsa_circ_0060927 and CYP24A1 in 50 leiomyoma tissues and adjacent normal tissues were evaluated using quantitative real-time PCR (qRT-PCRs) considering MED12 mutation profile.
Results: The results indicated the ectopic expression of hsa_circ_0060927 and CYP24A1 in 33.33% and 18.2% of uterine leiomyoma tissue samples, respectively; however, the expression of both transcripts were independent of the MED12 mutation profile in the leiomyoma samples.
Conclusion: Present results provide primary evidence for the role of circRNAs in the development of leiomyoma and also for the potential role of 1,25(OH)2D3 in maintaining the proper function of normal myometrium cells. Taken together, the results suggest that the dysregulation of vitamin D signaling and metabolic pathways may be involved in uterine leiomyomas tumorigenesis.
Key words: *Leiomyoma, Circular RNA hsa_circ_0060927, CYP24A1, MED12.*

P-78
Comparing developmental competence of mature and immature oocyte in PCOs patients and mRNA levels of *AMHR* gene as biomarker

Foroughmand A[1], Montazeri F[1, 2], Kalantar SM[2].

1.*Department of Biology, Faculty of Sciences, Shahid Chamran University, Ahvaz, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: smkalantar@yahoo.com

Background: In vitro maturation (IVM) could be a good choice for patients who are hormone sensitive and have few oocytes in stimulated cycles. Consequently, selecting embryos with the highest implantation potential is of great importance in assisted reproductive technologies (ART). To date, the choice of the best embryos to transfer is based on morphological parameters. Therefore, movement towards modern technologies such as genomics, transcriptomics, proteomics and metabolomics to select the most competent oocytes and/or embryos with the greatest implantation potential would be significant.
Objective: The aim was to assess the developmental competence of the in vitro and in vivo matured human oocytes as well as the *AMHR* gene expression of cumulus cells (CCs) between two groups in order to evaluate oocyte maturity in PCOs patient.
Materials and Methods: The oocytes and the associated CCs were retrieved from 27 PCO women and divided into groups of GV and MII according to the nuclear maturity status in order to studding developmental competence as well as expression patterns of *AMHR* gene using real time PCR.
Results: The fertilization and embryo formation rates were 61.5% and 84.5% vs. 67.1% and 90.8% in test and control groups, respectively. There were significant differences in mRNA levels of *AMHR* gene between the groups.
Conclusion: It seems that using immature oocytes could be helpful for patients at risk of ovarian hyperstimulation syndrome (OHSS) as the same as patients with diminished ovarian reserve.
Key words: *Polycystic Ovarian Syndrome, In vitro maturation, Oocyte maturity, Embryo selection, AMHR gene, Biomarker.*

P-79
Different expression of MUC1, FGF2, HBEGF, CSF1, VEGFA in the female reproductive tract in women with and without ectopic pregnancy

Golkar S[1], Afsharian P[2], Amjadi FS[3], Chekini Z[1], Ghaffari F[1], Aflatoonian R[1].
1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran Iran.*
3. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Email: r.aflatoonian@gmail.com

Background: Establishment of viable pregnancy requires embryo implantation and placentation. Ectopic pregnancy (EP) implantation junction breakdown occur outside of the uterine cavity with around 98% implanting in the fallopian tube. Among the genes effective in implantation these genes (MUC1), (FGF2), (HB-EGF), (VEGF-A), (CSF-l) are of particular importance. This study aims to investigate mRNA expression of MUC1, FGF2, HB-EGF, VEGF-A, CSF-l in fallopian tubes and endometrium of women who have EP compared with fallopian tubes and endometrium of pseudo-pregnant women. We hypothesize that expression of this genes in human fallopian tubes and endometrium may change during EP. Colony-stimulating factor 1 (csf1), a cytokine required for the differentiation, proliferation and survival of most macrophages, signals through a high-affinity receptor, csf1r, that is expressed on macrophages and their precursors. Fibroblast growth factors (FGFs) that signal through FGF receptors (FGFRs) regulate a broad spectrum of biological functions, including cellular proliferation, survival, migration, and differentiation. Mucins are high molecular weight (MW) glycoproteins, which contain at least 50% of carbohydrate O-linked to a threonine/serine rich peptide core. MUC1 functions include lubrication and protection against pathogens. Vascular endothelial growth factor (VEGF) is a potent antigenic factor responsible for vascular development that acts through its receptors. HBEGF is a member of the EGF family, which includes proteins that bind to tyrosine kinase receptors of the human EGF receptor (ERBB) family. HBEGF contributes to trophoblast survival and extra villous differentiation during early pregnancy, and is dysregulated in placental insufficiencies.
Objective: Our aim is to evaluate the expression of these genes (MUC1, FGF2, HB-EGF, VEGF-A, CSF-l) in the endometrium and fallopian tube between the ectopic pregnancy and the control group (pseudo pregnancy).
Materials and Methods: This was a case-control study. The case group consisted of women who underwent salpingectomy because of EP. The control group consisted of women with normal fallopian tubes that underwent hysterectomy. Prior to tubal sampling, each control subject received an injection of human chorionic gonadotropin (hCG) to produce a state of pseudo-pregnancy. Fallopian tubes and endometrial tissue from both groups were procured. We investigated VEGFA, MUC1, CSF1, FGF2 and HBEGF mRNA expressions in endometrial tissue and Ampulla section of the fallopian tube by Quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR).
Results: Our results showed expressions of these genes in endometrial tissue and Ampulla section of the fallopian tube in both groups. The expression of MUC1, HBEGF, and FGF2 genes in both groups (endometrial and ampulla) was higher in the case group than control. The expression of VEGFA and CSF genes in the endometrium of the case group (EP) was higher than the control group and showed higher expression in the Ampulla of the control group.
Conclusion: Different expression of current genes of EP groups in the fallopian tube and endometrium might have a role in the pathogenesis of embryo implantation in women who suffering from ectopic pregnancy. This investigation produce strong evidence of critical roles of molecular aspect which influences in fallopian tube implantation.
Key words: *Ectopic Pregnancy, Fallopian Tube, Endometrium, Gene Expression, MUC1, FGF2, HB-EGF, VEGF-A, CSF-l.*

P-80

Aberrant expression of microRNAs 16 and 21 and gene targets in women with unexplained recurrent miscarriage: A case-control study

Karami N[1], Mirabutalebi SHR[1], Kalantar SM[2].
1.*Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: kalantarsm@ystp.ac.ir

Background: Recurrent miscarriage (RM), as the occurrence of two or more of pregnancy loss before the 20th wk, can occur for multiple causes. One of the causes of miscarriage may be a defect in the process of angiogenesis because the delivery of nutrients to the fetus is decreased and it may also lead to miscarriage. Also, micro ribonucleic acids (miRNAs) play an important role in the development of diseases. The miRNAs 16 and 21 are the most well-known angiogenesis-related miRNAs, which their gene targets are vascular endothelial growth factor-A and phosphatase and tensin homolog, respectively.
Objective: To evaluate the changes in expression of miRNAs 16 and 21 and their association with the gene targets in women with unexplained RM.
Materials and Methods: In this case-control study, blood samples from 25 women with unexplained RM and 25 controls were taken. After extraction of RNA, the relative expression of miRNAs and their gene targets was measured using real-time quantitative reverse transcription-PCR method.
Results: Our findings showed that miR-21 expression was significantly decreased in both plasma and peripheral mononuclear cells (p=0.04 and p=0.02, respectively) and could be associated with the PTEN expression (p=0.03), however, there is no significant correlation between miR-16 and VEGF-A.
Conclusion: One of the most remarkable results of this study is that miR-21 showed significant changes in both plasma and peripheral mononuclear cells, which can be related to the etiology and progression of RM.
Key words: *Recurrent miscarriage, Angiogenesis, MiR-16, VEGF-A, MiR-21.*

P-81

Association of rs2234693 polymorphism with idiopathic male infertility

Mobasseri N, Nikzad H, Karimian M.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: mdkarimian@gmail.com

Background: Polymorphisms in estrogen receptor alpha gene could affect risk of male infertility.
Objective: in this study, we investigated the association of rs2234693 polymorphism in estrogen receptor alpha gene with idiopathic male infertility in an Iranian population.
Materials and Methods: In a case-control study, 439 subjects including 226 infertile men and 213 fertile men were enrolled. After blood collection and genomic DNA extraction, the rs2234693 genotyping was performed by PCR-RFLP technique.
Results: There were a significant protective association between rs2234693TT genotype and idiopathic infertility (OR=0.54, 95% CI=0.3-0.98, p=0.042). In addition, there are significant protective associations between rs2234693 polymorphism and both asthenozoospermia (OR=0.47, 95% CI=0.24-0.92, p=0.029) and non-obstructive azoospermia (OR=0.46, 95% CI=0.21-0.99, p=0.046). Also, allele analysis revealed a significant protective association between allele T and asthenospermia (OR=0.68, 95% CI=0.46-0.99, p=0.044).
Conclusion: These findings suggest that the ESR1-PvuII transition could be considered as a possible protective factor against male infertility.
Key words: *Idiopathic male infertility, Estrogen receptors, Genetic polymorphism, rs2234693.*

P-82

Identification of a novel mutation in MT-TC gene in patients with idiopathic recurrent miscarriage

Mojodi E[1], Moshtaghioun SM[1], Ghasemi N[2], Falahati A[1].
1.*Department of Biology, Faculty of Sciences, Yazd University, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: elham.mojodi68@gmail.com

Background: Recurrent miscarriage (RM) is three or more consecutive pregnancy losses prior to 20 wk from the last menstrual period. There exist a small number of accepted etiologies for RPL: Genetic Factors; Anatomic Factors; Autoimmune and Endocrine Factors. Transfer RNA (tRNA) genes are essential components of protein biosynthesis in mitochondrion. These genes are hotspots for mutations. These mutations are associated with a wide spectrum of human diseases.
Objective: The aim of this study was Identification of mitochondrial tRNA Cysteine gene mutations in Iranian patients with idiopathic recurrent miscarriage.
Materials and Methods: We investigated possible mutations in the MT-TC gene in 100 women with idiopathic RM and 100 healthy controls by PCR-SSCP and sequencing. RM in our patients diagnosed by specialists from infertility center of Yazd, Iran. Then sequencing results analysed by bioinformatics websites and software such as Mamit-tRNA; Mito map; RNA Fold, Gene Runner and Mega4.
Results: In 4 patients we found a new mutation 5762delA that cause change in tRNA secondary structure. This mutation is novel and has not previously been reported in any other disease.

Conclusion: The information provided by Mamait-tRNA and RNA fold website's databases, showed that this mutation can lead to changes in RNA secondary structure. According to Mamait-tRNA database, this mutation is in ACC domain, a preserved region in all tRNAs. RNA fold results also show a decrease in free energy indicating more stability of the mutated tRNA. Based on these results, the 5762delA mutation may be considered as a possible susceptibility factor for RM disease. More studies of mitochondrial tRNAs altogether in a larger population needed for accurate judgment.

Key words: *Recurrent miscarriage (RM), Mitochondrion, MT-TC gene.*

P-83

The frequency of rs14035 polymorphism of *RAN* gene and its association with unknown recurrent pregnancy loss in women referred to Yazd Reproductive Sciences Institute

Mortazavifar ZS[1], Ashrafzadeh HR[2], Seifati SM[1], Ghasemi N[2].

1.*Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran.*

2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: n479g@yahoo.co.uk

Background: Spontaneous abortion occurs in 15% of clinical pregnancies before the 20th week of gestational age. Repeated pregnancy loss (RPL) is defined as two or more spontaneous abortion.

Objective: It is indicated that genetic factors are responsible for 5% of RPLs. *RAN* gene is a member of the Ras superfamily. The mutation of *RAN* gene inhibits DNA synthesis and induces the altered gene expression in the uterus. Therefore *RAN* mutation may be involved in the mechanism of RPL. In this study, we assessed the prevalence of "rs 14035" polymorphism of *RAN* gene in women with unknown RPL and also compared it with fertile women without the history of RPL.

Materials and Methods: In the case-control study, 100 women with unknown RPL before the 20th wk of gestational age were recruited. The women were 20-40 yr old and their husbands were identified with normal karyotype in addition to normal sperm parameters. The control group included 100 women with regular menstrual cycles, who had at least one live birth and no history of abortion. The prevalence of "rs 14035" polymorphism of *RAN* gene was investigated using PCR-RFLP technique.

Results: Frequencies of the TT, TC, and CC genotype of *RAN* gene polymorphism in patients were 9%, 40%, and 51%, respectively while frequencies in control were 11%, 38%, and 51%, respectively. There was no significant differences between the genotypes in two groups (p=0.882).

Conclusion: According to our study, rs14035 polymorphism of *RAN* gene appears to have no association with the risk of unknown recurrent pregnancy loss in the studied population. However, additional studies, especially with larger sample size are needed to validate these findings.

Key words: *RAN gene, rs14035polymorphism, RPL, PCR-RFLP.*

P-84

Chromosomal aberrations in pregnancy loss: insight on the effect of consanguinity, review of 1625 cases, comparison with karyotype

Najafi K[1], Gholami S, Moshtagh A, Bazrgar M, Sadatian N, Abassi G, Rostami P, Khalili S, Babanejad M, Nourmohammadi B, Faramarzi Garous N, Najmabadi H, Kariminejad R.

Department of Cytogenetics, Kariminejad-Najmabadi Pathology and Genetic Center,

Email: kimianajafi@yahoo.com

Background: Pregnancy loss affects 10-15% of pregnancies and is caused by several factors, maternal and fetal. Traditionally karyotype was used to detect the cause of abortion. Recently with the advent of array comparative genomic hybridization (a-CGH) has become a more accurate alternative for the detection of fetal chromosome anomalies. We are reporting the results of our 7-year experience and a comparison of the results from a-CGH with our twenty-year experience with karyotyping

Objective: In addition, we are comparing the rate and frequency of chromosomal aberrations in consanguineous couples with non-consanguineous couples.

Materials and Methods: DNA was extracted from 1625 product of abortions. In 1104 of cases both quantitative fluorescent-polymerase chain reaction (QF-PCR) and a-CGH and in 521 cases only a-CGH was performed.

Results: Detection rate using QF-PCR and a-CGH is 20% compared to 12.7% overall and 15.7% excluding failed samples by karyotypes in our center. QF-PCR and a-CGH failed in 1.9% of cases, while the failure rate for karyotypes was 20.1%. The difference of detection rate and failure rate is significant ($p<0.001$ and $p<0.001$ respectively). Unexpectedly we found a significant difference in frequency of imbalances in related vs. unrelated couples ($p<0.001$).

Conclusion: It is highly likely that the pregnancy loss in the consanguineous couples is caused by other genetic and immune mechanisms. It is plausible through the same mechanism by which single gene disorders have a higher prevalence of manifesting disease in consanguineous couples; they can cause lethal genetic disorders leading to pregnancy loss in these couples. We propose that further study and reports of other cohorts with similar consanguinity frequency is necessary to verify these findings.

Key words: *Miscarriage, Array comparative genomic hybridization, Consanguinity, Chromosomal abnormality, Recurrent abortion.*

P-85

Investigation of mitochondrial tRNA leucine (UUR) gene mutations in Iranian patients with idiopathic repeated pregnancy loss

Najimi Ali Abad P[1], Moshtaghioun SM[2], Ashrafzadeh HR[3].
1.*Department of Biology, Islamic Azad University, Ashkezar Branch, Yazd, Iran.*
2.*Department of Biology, Faculty of Science, Yazd University, Yazd, Iran.*
3.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: Najimiparisa@gmail.com

Background: Mitochondrial transfer RNAs (tRNA) genes are essential components of protein biosynthesis in mitochondrion. These genes are hotspots for mutations. These mutations are associated with a wide spectrum of human diseases. Many genetic factors are known to involve in repeated pregnancy loss (RPL).
Objective: The aim of this study was analysis of tRNAleu (UUR) possible mutations in women with RPL.
Materials and Methods: The nucleotide variations of tRNAleu UUR were investigated in 100 women with idiopathic repeated pregnancy loss. The related mitochondrial area was amplified using polymerase chain reaction (PCR). The PCR products were demonstrated by 2% agarose gel electrophoresis. Then all PCR products were run on SSCP gels, and samples showed considerable shifts due to nucleotide variations were sent for DNA sequencing. Sequencing results in order to further information, studied under bioinformatics websites and software.
Results: The sequence analysis revealed no mutations in tRNAleu.
Conclusion: Further study of an expanded series of these tRNAs mutations in a larger population sample is recommended to describe their possible etiologic role in idiopathic RPL.
Key words: *Mitochondria, Mutation, Repeated pregnancy loss, mtDNA, tRNAleu (UUR), PCR-SSCP.*

P-86

Study the association of two key polymorphisms in *H2BFWT* gene with idiopathic male infertility

Parvaresh L, Karimian M, Mazoochi T.
Department of Medicine, Kashan University of Medical Sciences, Kashan, Iran.
Email: taherehmazoochi@gmail.com

Background: *H2BFWT* is one of the testis-specific histones that plays an important role in spermatogenesis process and single nucleotide polymorphisms (SNPs) in this gene may lead to male infertility.
Objective: In this study, the relation of -9 C>T and 368 A>G transitions in *H2BFWT* gene with male infertility has been investigated in Kashan population.
Materials and Methods: In a case-control study, the blood sample was collected from 232 subjects including 109 patients with idiopathic male infertility and 123 fertile men. The genotypes of -9 C>T and 368 A>G polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.
Results: Our data showed that alleles -9T (95%CI: 1.75, OR: 1.04-2.95, p=0.035) and 368G (95%CI: 1.71, OR: 1.02-2.89, p=0.042) are associated with male infertility. Also, subgroup analysis revealed that -9 C>T polymorphism was associated with azoospermia while 368 A>G was associated with oligozoospermia.
Conclusion: Based on our findings, 368 A>G and -9 C>T polymorphisms can be considered as genetic risk factors for infertility in the male population of Kashan.
Key words: *Genetic polymorphism, H2BFWT gene, Male infertility, SNP genotyping.*

P-87

The prevalence of common mutations in thrombophilic patients in Iranian population with recurrent abortion

Pazoki N[1], Naseri F[2].
1.*Department of Cell and Molecular Biology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.*
2.*Department of Genetics, Faculty of Basic Sciences, East Tehran Branch, Islamic Azad University, Tehran, Iran.*
Email: nasrinpazooki@yahoo.com

Background: To date, several factors have been reported in recurrent miscarriage. Genetic mutations are the most important factors in women. Fetal thrombotic vasculopathy is a new described placental alteration with varying degrees of involvement and often associated with adverse prenatal outcomes. The diagnosis is made histologically and so is postnatal, which makes it a challenge in clinical practice. The aim of the present study is investigation of the common mutations in women with recurrent spontaneous abortion.
Objective: A cross-sectional study was conducted on 100 women with a history of recurrent miscarriage fetus in 2018.
Materials and Methods: In these patients, several genes such as MTHFR, F2, F5 Leiden, PAI1, F13 and FGB were analyzed by Tetra Arms-PCR and sequencing techniques. The most common mutations in these genes were sequenced and analyzed.
Results: According to the statistical results conducted in populations, MTHFR gene (C677T, A1298C) has the highest rate (50%) of common mutations (p=0.001). After that F2 (G20210A) and F5 Leiden (G1691A) have the highest statistical values (each one 20%). In addition to these genes, there are other unknown mutations which have not been studied in terms of Pathogenicity. Other genes have a smaller percentage of aborted fetuses infrequently.
Conclusion: Common polymorphisms in the thrombophilic system are likely to results in abortion in these subjects, due to impaired coagulation of the mother and the fetus. Investigating the presence of common

mutations and examining their association with other mutations in the thrombophilia as a prognostic in patients with recurrent abortions is necessary.

Key words: *Recurrent miscarriage, Thrombophilia's factors, Genetic mutations.*

P-88

Exome sequencing reveals *SYCE1* mutation associated with autosomal recessive azoospermia in an Iranian family; the second report in the worldwide

Rahimi Bidgoli M[1], Alavi A[1], Javan Parast L[1], Pashaei M[1], Fatehi F[2].

1. *Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.*
2. *Department of Neurology, Tehran University of Medical Sciences, Tehran, Iran.*

Email: afaghalavi@gmail.com

Background: 30-55% of couples' infertility is due to male infertility. Azoospermia which is characterized by absence of sperm in the ejaculate, accounts for 10-15% of infertility in the males and generally affects 1% of this population. Azoospermia is confirmed using endocrine tests and urologic and genetic analyses. Currently the most common genetic causes of azoospermia are; mutations in *CFTR* gene, chromosomal abnormalities and microdeletions of chromosome Y.

Objective: Identification of disease-causing gene in a large Iranian pedigree affected to azoospermia.

Materials and Methods: Consent form was signed. DNA was isolated from peripheral blood leukocytes of proband and family members of a consanguineous Iranian family affected to azoospermia. Whole exome sequencing (WES) was done on proband. Preliminary filtering of sequence variations was done to identify all nonsynonymous, stop-gain, stop-loss, deletion, insertion, and splice sites homozygous variants present in the proband. Subsequently, variations with a MAF >0.01 in public databases (1000Genomes, ExAc, ESP, GnomAD, Iranome, HEX, SISu and GME-Variome) were removed to find the probable disease-causing variations. Candidate variants were PCR-amplified and sequenced by Sanger method subsequently checked in family members in order to co-segregation analysis.

Results: This approach led us to identify a novel splice site variation c.375-2A>G in SYCE1 in the proband and this variant co-segregated with the disease status in the family and was not found in 200 ethnically matched controls or in 100 in-house control exomes and Iranome database.

Conclusion: Our study resulted in the second report of azoospermia with mutation in SYCE1 gene in worldwide. Mutation in this gene has been previously reported only two times: in two sisters from a consanguineous Arab family from Israel with primary ovarian failure (2014) and in two affected brothers from a consanguineous Jewish family with azoospermia (2015). SYCE1 encodes Synaptonemal complex (SC) central element 1 protein which plays an essential role during meiosis and its significance was previously demonstrated in a mutant mouse model so that male and female Syce1 null mice were infertile. Our variation resulted in RNA mis-splicing of SYCE1, and a premature stop codon. So, this variation can be resulted in inhibiting of synapsis initiation and consequently, completion of meiosis. Our results emphasize the importance of SC proteins in spermatogenenesis and suggests that a low percentage of infertility cases may be due to mutations in SC genes. The characterization of these mutations, together with available functional studies, will enable a deeper understanding of the underlying molecular bases for some of the infertile cases.

Key words: *Azoospermia, Splice site variation, SYCE1, Synaptonemal complex, Whole exome sequencing.*

P-89

Y chromosome microdeletion in infertile men with nonobstructive azoospermia (NOA)

Rezaei F, Shafiei M, Galehdari H.

Department of Genetics, Biology Faculty, Shahid Chamran University, Ahvaz, Iran.

Email: rezayifaride@gmail.com

Azoospermia is an infertility essential factor in men and present in approximately 15% of infertile men. Azoospermia Factor (AZF) located in the long arm of Y chromosome (Yq11). This locus is separated into three nonoverlapping subregions called AZFa, AZFb, and AZFc. These regions are essential for normal spermatogenesis and microdeletions in these regions are correlated with various alterations in spermatogenesis. Polymerase chain reaction (PCR) and sequence-tagged sites (STS) analysis are using for Y chromosome microdeletions (YCMD) in infertile men. Microdeletions in each of regions cause disorders. AZFa microdeletions cause Sertoli Cell-Only Syndrome, (SCOS), AZFb microdeletion is responsible for maturation arrest at meiosis (MA) and AZFc microdeletion are detected in patients with hypospermatogenesis (HP). Infertility is defined as failure that affects about 10-15% of couples worldwide in the half of these cases, male-related factors are responsible, therefore, the major causes of male infertility is azoospermia. YCDM screening for correct diagnosis of male infertility is necessary.

Key words: *Y chromosome microdeletion, Nonobstructive azoospermia, Infertility.*

P-90

The role of circle RNAs (circRNAs) in male fertility

Rezaei F, Shafiei M, Galehdari H.

Department of Genetics, Biology Faculty, Shahid Chamran University of medical Sciences, Ahvaz, Iran.

Email: rezayifaride@gmail.com

CircRNAs as a subclass of ncRNAs have appeared as a novel RNA of interest in gene regulation. It is estimated that 15996 circRNAs are transcribed in human testis. It is

found that these circRNAs have an important role in sperm production. Since SRY gene in adult was transcribed as a circle form (circSry), suggested that circRNAs has special function in sperm production. CircSry is expressed only in testis, suggested that it has a function as miRs sponge. This article was performed by searching on electronic databases including: PMC and nature. With using high-throughput sequencing, it is predicted that about 100000 circRNAs exist in humans that 15996 of them are expressed in testis and 14033 circRNAs are related to 5928 host genes. 70.6%, 13.0% and 9.8% of the expressed circRNAs in testis were derived from exonic, intergenic and intronic regions, respectively. Although there are a large number of circRNAs in the testis, but studies about them have just begun. These circRNAs may have important role in post-transcriptional regulation of spermatogenesis. Therefore, it is proposed that the circRNAs in testis may be used as novel biomarkers for male fertility.

Key words: *Circle RNAs, Male fertility.*

P-91

Multiplex PCR screening of Y-chromosome microdeletions in azoospermic ICSI candidate men

Sabbagh Nejad Yazd S[1], Sheikhha MH[2].

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

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

Email: roghayehkarimi21@yahoo.com

Background: It has been hypothesized that Y-q microdeletion can account for significant proportion of infertility in men. There are three nonoverlapping regions referred to as the "azoozpermia factors" AZFa, AZFb, and AZFc from proximal to distal part of Y-q. These have been defined as spermatogenesis loci. This region deletions have been shown to be involved in male azoospermic or severe oligoozospermic infertility.

Objective: Evaluation the rate of Y-chromosome microdeletions in infertile men.

Materials and Methods: In this case-control study, 25 azoospermic infertile men candidate for intracytoplasmic sperm injection (ICSI) were selected as case group. For control group, 25 normoozoospemric men were selected. All cases and controls had normal 46XY karyotype. DNA extraction and molecular analysis were done on blood samples. Multiplex-PCR method was done to identify the presence of microdeletion in AZFa, AZFb or AZFc loci. Eight STS primers that include two controls were selected to determine Y-chromosome microdeletions.

Results: 20% (5/25) of all patients have at least one microdeletion in more than one region of AZF loci. Totally 17 microdeletions was observed, one case had deletions in three AZF regions, and 4 cases had deletions in two AZF regions. The rate of deletions was 42% (7/17) for AZFc, 35% (6/17) for AZFa and 23% (4/17) for AZFb.

Conclusion: The molecular DNA analysis could help us to know the real cause of infertility and can give good information for good decision for example in men with microdeletions who want to undertake ICSI procedure the deletions will be passed to their son.

Key words: *Male infertility, Multiplex PCR, Y chromosome microdeletions.*

P-92

Evaluation of serum levels and genetic variation of soluble leptin receptor (GLN223ARG) in patients with unexplained infertility and fertile women

Tafvizi F[1], Ashrafi Sh[1], Ashrafzadeh HR[2].

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

2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: farzanehtafvizi54@gmail.com

Background: The soluble Leptin receptor (sOB-R) is the most important protein which binds to leptin and can influence on bioactive free leptin. Gln223Arg polymorphism was associated with obesity and could be a potential factor for infertility.

Objective: The aim of this study was to compare serum sOB-R concentrations in order to find the role of leptin receptor in unexplained infertility and the genetic variation of the leptin receptor gene in unexplained infertile women.

Materials and Methods: The subjects were 102 women with Unexplained infertility and 112 fertile women who met the inclusion criteria (FSH ≤10, AMH and normal prolactin and Age <40 yr). sOB-R was measured using ELISA method. Genotyping of Gln223Arg polymorphism was performed using RFLP-PCR methods.

Results: There were two-fold increases of sOB-R in healthy women than patients' group (p=0.001). There was no significant difference between the observed genotypes. Our finding represents no correlation between Gln223Arg polymorphism and unexplained infertility in women. The increased risk of obesity was observed in GG genotype than other genotypes (p=0.024). The studied populations were in equilibrium in the gene location.

Conclusion: According to these results, it seems that the amount of leptin in serum will be decreased by increasing the serum sOB-R. Thus, increased sOB-R level in serum can prevent leptin inhibiting effects on infertility. A decreased sOB-R level in serum of infertile women could be influenced on the inhibitory reaction of leptin in infertility.

Key words: *sOB-R leptin receptor, Unexplained infertility, GLN223ARG polymorphism.*

P-93
Amniocentesis following positive first trimester combined screening: A comparative study

Yassaee F[1], Shekarriz-Foumani R[2], Sadeghi S[3].
1.*Department of Obstetrics and Gynecology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2.*Department of Community Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3.*Department of Electrical- Biomedical and Mechatronics Engineering, Islamic Azad University, Qazvin Branch, Qazvin, Iran.*
Email: reza.shekarriz@gmail.com

Background: The birth of a neonate with chromosomal abnormalities e.g. Down syndrome has very serious problems for family, society and for the neonate itself and therefore prenatal evaluation is imperative in determining the fate of the fetus.
Objective: This research aims to assess the association and accuracy of amniocentesis with first trimester combined screening.
Materials and Methods: In this study, specimens from 1066 cases were analyzed for free Beta human chorionic gonadotropin, pregnancy associated plasma protein A, along with nuchal translucency and nasal bone ultrasonography from October 2013 till November 2014. Upon observing positive screening, mothers underwent amniocentesis. Finally the amniocentesis results were compared with that of first trimester screening.
Results: Our results determined a direct relation between the high age of the mother and gravidity with p=0.001 and p=0.020 with positive first trimester screening. Our study attained a 92% accuracy rate of amniocentesis due to one case of mosaicism of trisomy 21, that was not diagnosed, because it was not requested by physician. Only 12 (17.1%) cases out of 70 (mothers with positive first trimester screening) showed positive amniocentesis which had a significant relationship with chromosomal abnormality.
Conclusion: First trimester combined screening have very high accuracy (94.6%) in prediction of genetic abnormalities. The probability of positive first trimester screening is directly influenced by number of factors including the mother age and gravidity. Amniocentesis is necessary for all of mothers with positive first trimester screening and will almost always detect chromosomal abnormalities.
Key words: *Pregnancy first trimester, Amniocentesis, Prenatal diagnosis.*

P-94
Identification of mitochondrial tRNA leucine (CUN) gene mutation in Iranian patients with idiopathic recurrent miscarriage

Zakizadeh N.
Department of Biology, Islamic Azad University, Ashkezar Branch, Yazd, Iran.
Email: nasrin.7058@gmail.com

Background: Mitochondrial transfer RNAs (tRNA) genes are essential components of protein biosynthesis. These genes are hotspots for mutations. These mutations are associated with a wide spectrum of human disease. Many genetic factors are known in assessment of repeated pregnancy loss (RPL).
Objective: The aim of this study was identification of mitochondrial tRNA leucine (CUN) gene mutations in Iranian patients with idiopathic recurrent miscarriage.
Materials and Methods: The nucleotide variations of Leucine (CUN) and Histidine were investigated in 100 women with idiopathic repeated pregnancy loss. The related mitochondrial area was amplified using polymerase chain reaction (PCR). The PCR products were demonstrated by 2% agarose gel electrophoresis, all PCR products were run on SSCP gels and the samples showed considerable band shifts due to possible mutations were sent for DNA sequencing. Nucleotide variations were found in sequencing data, studied for further information under bioinformatics websites and software.
Results: The sequence analysis revealed 2 mutations in tRNAleu and 3 mutations in tRNAhis. These mutations were 12308A>G in 2 cases and 12192A>G in 1 case, as the most common mutations. Also, the results of tRNAhis sequencing showed the 12172G>A in 2 cases in 2 women as a novel mutation.
Conclusion: These tRNAs mutations can alter their steady state level and affect the structure of tRNAs. They may result in protein synthesis defects and, in turn, mitochondrial dysfunction. The mutations of these genes may help in the assessment of RPL. Further study of an expanded series of these tRNA mutants in a larger size population, is recommended to describe their etiologic role in idiopathic RPL.
Key words: *Repeated pregnancy loss, Mitochondrion, Mutation, tRNAleu (CUN), PCR-SSCP.*

P-95
Protective association of -308G/A transition in *TNFa* gene with azoospermia

Zamani-Badi T, Karimian M, Nikzad H.
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Email: hnikzad10@gmail.com

Background: Male infertility is a multifactorial problem in which anatomic, environmental, lifestyle, physiological and genetic factors can be influential. Genetic polymorphisms in some cytokines gene families including tumor necrosis factor alpha (*TNFa*) may be associated with susceptibility of male infertility. Several single nucleotide polymorphisms (SNPs) are studied in the promoter region of *TNFa* such as -1031T/C, -863C/A, -857C/T, -575G/A, -376G/A, -244G/A, -238G/A, and -308G/A. Among these variations, the -308G/A polymorphism could affect the male infertility risk.
Objective: The aim of this study was to investigate the association of -376G/A in *TNFa* gene with idiopathic male infertility in an Iranian population.

Materials and Methods: In a case-control study, 200 subjects including 100 fertile men and 100 infertile men were enrolled. After blood samples collection, genomic DNA was extracted and -376G/A genotyping was performed by PCR-RFLP method.
Results: Our data revealed that there is no significant association between -308G/A transition and male infertility in overall analysis. However, we found a significant association between -308G/A polymorphism and decreased risk of azoospermia (OR=0.1344, 95% CI=0.0207 to 0.8725, p=0.0355).
Conclusion: Our results suggest that the -308G/A polymorphism may be a protective factor against azoospermia.
Key words: *Male infertility, Azoospermia, Tumor necrosis factor alpha, Genetic polymorphism.*

4- Infertility, Gynecology

P-96
Effects of flavonoid Genistein on liver enzymes and ovary tissue modification in the treatment of polycystic ovary syndrome

Alivandi Farkhad S, Khazali H.
Department of Physiology, Faculty of Biological Science and Technology, Shahid Beheshti University, Tehran, Iran.
Email: sa_farzad2002@yahoo.com

Background: Polycystic ovarian syndrome (PCOS) is the most common reproductive endocrine disease with a prevalence of 5-10% in women of reproductive age. The clinical features of PCOS are heterogeneous and vary in intensity. Oligomenorrhea or amenorrhea contributing to chronic anovulation and infertility are the most frequent conditions observed. PCOS is also associated with several metabolic disturbances which characterize metabolic syndrome such as obesity, dyslipidemia and insulin resistance. More recently, increased prevalence of Nonalcoholic Fatty Liver Disease (NAFLD) has been reported in patients with PCOS. Interestingly, flavonoids have been found to have positive effects on lipid metabolism, insulin resistance, inflammation, and oxidative stress, the most important pathophysiological pathways in NAFLD.
Objective: The aim of this study was to investigate the therapeutic effects of genistein on liver enzymes and ovarian histology in rats with estradiol valerate (EV)-induced PCOS.
Materials and Methods: In this study the syndrome induced by muscular injection of 40 mg/kg Estradiol Valerate to 32 female Wistar rats, weighing 170-180 g. Control group receive no injection. After 60 days the animals divided to control, PCOS and PCOS treated with Genistein (2 and 5 mg/kg) groups. After 21 days intraperitoneal treatment of Genistein, the ovaries of all groups histologically studied and blood samples were collected and Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured.
Results: The levels of AST and ALT in treatment groups with Genistein 5 mg/kg were significantly reduced while compared to the PCOS groups. There was a signification polycystic improvement in ovaries treated with high concentration of Genistein in comparison with PCOS group and reduction of cystic follicular number and corpus luteum increase were found, that could be a sign of renewed ovulation.
Conclusion: Genistein is capable of producing beneficial reproductive effects offering an optimal treatment approach for patient with NAFLD, PCOS and infertility. Additionally, flavonoids have fewer side effects compared to standard drug therapies. It is possible that, this ability is because of Genistein antioxidant and anti-inflammatory effects that motivate reduction of liver enzymes and cysts number and natural development of follicles.
Key words: *Genistein, Polycystic ovary, Liver enzymes, Infertility.*

P-97
Assisted reproductive technology (ART) and birth weights

Bagheri N[1], Mohammadi P[1, 2, 3], Eshaghi Sh[1], Torkiyan N[1], Fadaee M[1], Naghshineh E[1, 4].
1.*Maryam Infertility Center, Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
2.*Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
3.*Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
4.*Department of Obstetrics and Gynecology, Infertility Center, Beheshti University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran*.
Email: nasrinbagheri1396@gmail.com

Background: Recently there has been an increased focus on epigenetic insults in utero predisposing to adult disease. Low birth weight may be a marker for such prenatal insults. At the same time, a growing body of evidence suggests that children conceived through assisted reproductive technologies (ART) are at increased risk for poor perinatal outcomes, including low birth weight.
Objective: The goal of our study was to investigate birth weight outcome of singleton children conceived using ART.
Materials and Methods: All singleton children conceived using ART at the Maryam infertility center in Beheshti Hospital in Isfahan between January 2017 to June 2018 were studied. Each case child conceived with ART was matched with multiple controls based on plurality, maternal age, year of birth. Data collected included birth weight, maternal characteristics such as age, antepartum complications such as diabetes and preeclampsia, type of delivery (operative or not), and gestational age at delivery. Birth weight was analyzed as a continuous variable in grams.

Results: Comparison of duration of pregnancy and birth weight of infants born after infertility treatment confirms a shorter pregnancy span and a lower mean birth weight in infants born after IVF and ICSI. If women with pregnancies after ART deliver before term, neonatal birth weight is significantly lower.
Conclusion: There is a specific effect of ART, mainly IVF and ICSI, on both shortening the duration of pregnancy and lowering neonatal birth weight. Both these parameters seem to be interrelated consequences of some modification in the gestational process induced by the infertility treatment. Freezing and thawing of oocytes in the pronucleate stage had a lesser impact on pregnancy span and on neonatal birth weight.
Key words: *Birth weight, Singleton, IVF, ICSI, Outcome.*

P-98

The risk of hypertension in polycystic ovary syndrome: long-term population-based cohort study

Behboudi- Gandevani S, Ramezani Tehrani F, Noroozzadeh M, Rostami Dovom M, Farahmand M, Amiri M, Azizi F.
Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: fah.tehrani@gmail.com

Background: Polycystic ovary syndrome (PCOS) is one the most common endocrinopathies associated with metabolic complications, Whether PCOS is associated increased risk of hypertension with independent of potential confounders' remains controversial.
Objective: The aim of this study was to evaluate the hazard of hypertension in women with polycystic ovary syndrome (PCOS) comparing with control group of healthy women.
Materials and Methods: This study was prospective cohort study with the median and interquartile range of 12.9 (10.8-13.9) yr. The participants of this study was selected from among women participating in Tehran Lipid and Glucose Study. A total of 1,702 reproductive-age women including 178 women with PCOS and 1,524 controls were recruited. The main outcome measure was cumulative incidence of hypertension using the Kaplan-Meier method and compared using the log-rank statistic; univariate and multiple extended Cox proportional hazards regression with age as the time scale was used to estimate the adjusted hazard ratio (HR) of developing hypertension.
Results: The incidence rates of hypertension were 13.9, per 1,000 person-years for women with PCOS; and 13.8 per 1,000 person-years for the healthy control women. Women with PCOS aged lower than 40 years had an adjusted higher risk of developing hypertension (HR 2.08; 95% confidence interval, 1.0-3.9), but the risk disappeared after age 40.
Conclusion: In this long-term population-based cohort study, the risk of developing hypertension in young women with PCOS was higher than in controls, but these risks were diluted in the late reproductive period.
Key words: *Polycystic ovary syndrome, Population-based cohort study, Hypertension.*

P-99

Comparison of sitagliptin and metformin on the anti-mullerian hormone level in infertile women with polycystic ovarian syndrome in IVF cycle

Daneshjou D[1], Soleimani Mehranjani M[1], Zadeh Modarres SH[2].
1.*Department of Biology, Arak University, Arak, Iran.*
2.*Department of Obstetrics and Gynecology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Email: d.daneshjou@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among adult women in the developed world. PCOS is not a specific endocrine pathology with a solitary cause, but a syndrome with different signs and symptoms. Anti-mullerian hormone (AMH) acts as a regulator of folliculogenesis. The level of serum AMH in PCOS were evaluated about two or three-fold. Serum AMH was recently proposed as diagnostic marker of POCS. Metformin is the most common antihyperglycemic agent, recently introduced for treatment of women with PCOS. Some studies have attempted to investigate the AMH serum level during treatment with metformin in patients with PCOS. Some clinical trials of PCOS have shown effects of Sitagliptin in women with PCOS.
Objective: The aim of this study was to compare effects of Sitagliptin and Metformin on AMH levels in PCOS patients suffering from infertility.
Materials and Methods: In this clinical trial, 60 infertile women, aged 23-38 yr, diagnosed with PCOS according to the Rotterdam 2003 criteria and candidate for IVF. Patients divided into 3 groups for receiving Sitagliptin, Metformin and placebo every day for 8 weeks (n=20 each group). The serum AMH and BMI level were recorded before start of stimulation and after 8 weeks of treatment. We used SPSS version 19 for data analysis.
Results: The level of AMH in sitagliptin group and metformin groups was significantly lower than the placebo group. AMH level in the sitagliptin group was significantly lower than the metformin group.
Conclusion: In this clinical practice, reducing serum AMH levels of women with infertility and PCOS treated with Sitagliptin and metformin can show better results in IVF cycle. The AMH levels on Sitagliptin group was observed in patients with PCOS comparable to metformin group.
Key words: *Polycystic ovarian syndrome, Anti-mullerian hormone, Metformin, Sitagliptin.*

P-100

Sitagliptin ameliorates anti-mullerian hormone level in infertile women with polycystic ovary syndrome

Daneshjou D[1], Soleimani Mehranjani M[1], Zadeh Modarres SH[2], Shariatzadeh SMA[1].
1.Department of Biology, Arak University, Arak, Iran.
2.Department of Gynecology and Obstetrics, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: d.daneshjou@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is a common hormonal imbalance among women in reproductive age and is the main cause of their infertility due to anovulatory. PCOS is not a specific endocrine disorder with a single cause, but is a syndrome with different signs and symptoms. Anti-mullerian hormone (AMH) is a regulator hormone in folliculogenesis and its serum level increases about two or three folds in PCOS patients relative to healthy women. Sitagliptin, as an oral antihyperglycemic agent uses in treatment of diabetes mellitus type 2, is considered recently in clinical investigations of PCOS.
Objective: The aim of this study was to evaluate the effect of sitagliptin on the level of AMH in PCOS patients suffering from infertility.
Materials and Methods: In this clinical trial, 40 infertile PCOS patients were selected based on the Rotterdam criteria; Then they divided into 2 groups (n=20): sitagliptin group (treated with 50 mg of sitagliptin/ two times per day) and placebo group. Oral treatment was carried out for 8 weeks and serum level of AMH was measured in the groups before and after treatment. Data was analyzed statistically using one-way ANOVA and Tukey's test and means difference was consider significantly different at $p<0.05$.
Results: Serum AMH level was significantly decreased after treatment with sitagliptin (5.41±1.09 vs. 7.17±1.56).
Conclusion: Our obtained result from this clinical study showed that sitagliptin can decrease the serum level of AMH in women with infertility due to PCOS. Therefor it seems that sitagliptin has therapeutic efficiency for treatment of PCOS.
Key words: *Polycystic ovary syndrome, Anti-mullerian hormone, Sitagliptin.*

P-101
Investigating the toxicity of carbon nanostructures on reproductive systems: A systematic review

Dehghanbaghi N[1], Zare-Zardini H[2].
1.*Department of Biology, Faculty of Science, Science and Art University, Yazd, Iran.*
2.*Hematology and Oncology Research Center, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: hadizarezardini@gmail.com

Background: Nanotechnology is a new scientific field with novel and small compounds that utilize in different fields over the past ten years especially in medicine. Between these nanostructures, carbon nanostructures are more considerable due to amazing properties. Beside suitable and benefit applications, carbon nanostructures have different side effects on normal cells, tissues and organs. In this review, we investigated the toxicity of carbon nanostructures on reproductive systems.
Objective: In this review, we investigated the toxicity of carbon nanostructures on reproductive systems.
Materials and Methods: In this systematic review, all article published on toxicity of carbon nanostructures were searched using PubMed, Google scholar, Science direct, Science Citation Index and Embase. Below key words were used for data search: Carbon Nanomaterials/nanostructures, Graphite nanoplatelets, Graphene, Graphene oxide, Single-walled carbon nanotubes, Multi-walled carbon nanotubes, Fullerene, toxicity, reproductive system, ovary, testis, sexual hormones, functionalization and toxicity, animal model, and human model. Inclusion criteria were as follows: all article about toxicity of nano carbon on all fields of reproductive sciences published between 2000- 2018.
Results: Totally, 265 articles were obtained based on criteria. After assessment of subject, titles and abstracts, 212 articles were excluded due to same article or similar data. So, 53 articles were selected and their data was carefully analyzed. This systematic review shows that all shapes of carbon nanostructures have potent toxicity on reproductive system, but between all carbon nanostructures, SWCNTs and Fullerene have the highest and lowest toxicity, respectively. Between different types of graphene, the sequence of toxicity was: Reduced grapheme, graphene, and graphene oxide. In all articles, it's mentioned that the most important mechanism for toxicity of carbon nanostructures is production of reactive oxygen species and oxidative stress. Articles also showed that male reproductive system is more sensitive than female's ones. The reason given for this difference was that unlike the other organs, testes don't have suitable protection mechanisms. Data form these articles showed that carbon nanostructures can lead to decrease of fertilization capacity by side effects on sexual hormones, reduction in number of leydig cells, sperm motility and viability, and induction of changes in ovarian tissue. In these investigated articles, functionalization has been introduced as the best procedure for reduction of toxicity on reproductive systems. The largest functional groups used for functionalization were: -COOH, -NH2, basic and acidic amino acids (Lysine, Arginine, Histidine, Glutamate, and Aspartate), and polyethylene glycol (PEG).
Conclusion: Based on this review, all allotrope of carbon nanostructures have moderate-high toxicity on reproductive systems in both males and females. So, this must be done with caution in using these nanostructures.
Key words: *Toxicity, Carbon Nanostructures, Reproductive Systems.*

P-102
Molecular evaluation of endometrial receptivity in women with congenital malformations of genital tract

Fallahi S[1], Hayati Roudbari N[1], Aghajanpour S[2], Ramazanali F[2], Moini A[2], Aflatoonian R[2].
1.*Islamic Azad University, Science and Research Branch, Tehran, Iran.*
2.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: r.aflatoonian@gmail.com

Background: Uterine congenital malformations (UCM) occur in 3-4% of women overall, in 4% of infertile women and in 15% of those who have experienced recurrent miscarriage. Uterine malformations consist of a group of miscellaneous congenital anomalies of the female genital system. Their mean prevalence in the general population and in the population of fertile women is ~4.3%, in infertile patients ~3.5% and in patients with recurrent pregnancy losses ~13%. Septate uterus is the commonest uterine anomaly with a mean incidence of ~35% followed by bicornuate uterus (~25%) and arcuate uterus (~20%). To present the potential clinical impact which uterine congenital malformations (UCM) may exert on human embryonic implantation and discuss in receptive endometrium genes.
Objective: In this study, differences in gene expression between UCM and normal endometrium were evaluated using a PCR Array approach.
Materials and Methods: The mRNA of endometrium was analyzed in 3 groups (septet uterus, unicorn uterus, normal uterus, n=15). The differentially expressed genes of receptivity endometrium pathway were analyzed.
Results: The mRNA levels of expression in EGF, CSF, HOXA10, HOXA11, LIF, LIFR, MUC1 were all significantly higher in the unicorn group compared to control ($p<0.05$) but EGF, CSF, HOXA10, HOXA11, LIFR, MUC1 were all significantly lower in the septate group compared to control ($p<0.05$). However, LIF was expressed higher in septate group compared to control ($p<0.05$).
Conclusion: This investigation showed that the patients with UCM have different pattern of endometrial receptivity. Further research should be done to clarify the endometrial receptivity in different type of uterine anomaly.
Key words: *Endometrium receptivity, Congenital malformations, PCR array, Uterine, Genital tract.*

P-103

A randomized comparative study of three different educational interventions prior to ovarian puncture, measuring preoperative anxiety

Farnia F[1], Aflatoonian A[2].
1. *Research Center for Nursing and Midwifery Care, Department of Nursing School of Nursing- Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: far.farnia@yahoo.com

Background: Surgery and infertility are stressful experiences for patients. Information provision has been considered to reduce adverse pre-operative anxiety - related effects. Therefore, a variety of educational interventions have been implemented to assist patients with surgery. But, the most effective approach to education has yet to be established.
Objective: The study was comparing the effectiveness of peer, nurse with physician education on pre-operative anxiety in infertile women.
Materials and Methods: In this randomized clinical trial, 198 eligible infertile women were randomized into three groups: the nurse, peer, or physician education. The patients were educated before their scheduled surgery. The Spielberger State-Trait Anxiety Inventory (STAI) was filled out by all participants for measuring the patient anxiety at the time of hospital admission and the prior to surgery. Participants in the nurse-educated and peer-educated groups received a group education program by a nurse or peer, respectively, after the initial completion of the STAI. The physician education group were educated at the time of admission and their discharge as a part of the routines care.
Results: The mean score anxiety was 44.47, 46.92, and 42.60 at the time of hospital admission and 39.38, 41.06, and 43.42, prior to surgery in the nurse, peer, and also the physician education patient groups, respectively. There was a significant difference in the mean score anxiety in each group before and after the intervention ($p<0.0001$). The difference among the groups was not significant.
Conclusion: The results of this study can provide an empirical basis for nursing care. Our findings demonstrate that Patients awaiting infertility surgery need supportive interventions to reduce anxiety. Although all education interventions reduce the pre-operative anxiety, it seems peer education is more effective. Hence, making benefit of the peer"s potential regarding the compensation for staff shortage for pre-operative education as well as investigating the effect of individual education is suggested for the further studies.
Key words: *Pre-operative anxiety, Nurse education, Infertile women, Physician, Peer.*

P-104

Effects of dehydroepiandrosterone (DHEA) on response of ovarian stimulation in IVF cycle in infertile women with diminished ovarian reserve: Before and after clinical trials

Farshidfar B, Zamaniyan M.
Department of Obstetrics and Gynecology Mazandaran University of Medical Sciences, Sari, Iran.
Email: blackart_blackart@yahoo.com

Background: One of the best therapies in women with diminished ovarian reserve (DOR), is the use of dihydroepiandrosterone (DHEA) before starting in vitro fertilization (IVF) cycles to increase ovarian reserve.
Objective: In this study, we aimed to compare the effect of DHEA on women with DOR below and above 35 years old and to see if it's useful at an earlier age.
Materials and Methods: This is a clinical trial study performed on 35 infertile women with DOR who referred to Imam Khomeini Hospital in Sari in 2017. The intervention was for six weeks, DHEA tablets were used before the start of the ICSI-ET cycle. Antral follicular counts (AFC) and serum AMH levels were measured before and after the intervention and then ICSI was performed. Changes in AMH levels and AFC, number and quality of ovum and embryos and pregnancy, and implantation and abortion rates were measured.

Results: The mean AMH levels (p=0.02) and AFC (p<0.001) after DHEA consumption were significantly different from those prior to administration of DHEA, and the increase in the AFC was more significant in the age group under 35 (p=0.03). Also, these changes were more significant in Body mass less than 25 kg/m^2 (p=0.04).
Conclusion: It can be concluded that probably the supplementation of DHEA in women with insufficient ovarian capacity improves IVF prognosis and other parameters including Antral follicular counts and AMH especially in women under 35. Studies have shown that the probability of pregnancy occurrence in women was lower with increased BMI.
Key words: *Dehydroepiandrosterone, Ovarian Reserve, Anti-Mullerian Hormone, Infertility, Female.*

P-105
The effect of anti-mullerian hormone for prediction of oocyte and improving pregnancy under ART

Ghiasi Hafezi S[1], Mangoli E[2], Eftekhar M[2], Ghamsary M[3], Karbassi SM[1].
1.*Department of Mathematics, Yazd University, Yazd, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Biostatistics and Epidemiology, Loma Linda, University, USA.*
Email: somayehghiyasi@gmail.com

Background: Infertility has many potential causes, which may involve the man, the woman or both partners. In some cases, no cause of the problem can be determined, in which case the infertility is described as "unexplained." Infertility can be distressing and devastating for many people. They will experience stress, sadness, or feelings of hopelessness. Clinical knowledge and advancement in technology has been helpful for infertility via Assisted Reproductive Techniques (ART). National guidelines and national registries for (ART) are becoming more widespread and are expected to play an important role in promoting best practice in ART in the future. Anti Mullerian Hormone (AMH) is one of the most accurate tests to assess a woman's ovarian reserve and is produced by the medium sized immature eggs in the ovaries. It is a powerful test because it gives an indication of likely menopausal age, likely reproductive time frame and likely response to drugs in an IVF cycle (how many eggs you are likely to get). AMH also can be used to confirm polycystic ovary syndrome and gives a guide to egg quality. However what AMH cannot tell you is whether you are going to have a baby.
Objective: To determine effects of Anti-Mullerian Hormone on the variables M2 (Metaphase II), 2PN (two pronucleia), Maturation rate oocyte and pregnancy rate in infertile women under Assisted Reproductive Technology (ART) (IVF, ICSI).
Materials and Methods: A sample of 1000 participants collected at Yazd Research and Clinical Center for Infertility, Yazd, Iran, from April 2016 to February 2017. The participants were divided into three groups of AMH (1: less than 1 ng/ml, 2: between 1 and 3.5 ng/ml 3: and greater than 3.5 ng/ml). Analysis was done by Chi-square, independent t test, ANOVA test and logistic regression.
Results: A two sample t test results shows that the mean age of women 29.5±5.0 for pregnancy is significantly lower than 31.50±5.8 for non-pregnancy (p<0.001). There is a statistically significant difference (p<0.001) between the three groups of AMH for the variables M2, Maturation rate oocyte and 2PN. Logistic regression of clinical pregnancy showed significant effect on AM (2 vs. 1, OR=1.51, CI (0.83, 2.76), 3 vs. 1, OR=3.06, CI (1.68, 5.56)) Fertilization (1 vs. 2, OR=1.56, CI (1.06, 2.31), 1 vs. 3, OR=1.83, CI (1.25, 2.70)) Female age OR=0.96, CI (0.93, 0.99). The Chi-square test revealed, the proportion of pregnancy tended to differ across AMH group (p<0.01).
Conclusion: Our analysis of data shows the higher level AMH on M2, TwoPN, Maturation rate oocyte is better prediction of the clinical pregnancy rate in infertile women. The Logistic regression also provide the evidence of prediction of clinical pregnancy rate on AMH, Fertilization ،Female age.
Key words: *Pregnancy rate, Assisted Reproductive Technology.*

P-106
Effects of hydro-alcoholic extract of VAC on OHSS complication in a rat model

Izadi M[1], Esmaeilidehaj M[2], Aliabadi A[2], Rezvani ME[2].
1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Physiology, School of Medical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: erezvani@yahoo.com

Background: Ovarian hyperstimulation syndrome (OHSS) is a serious complication of ovarian stimulation that lead to ovarian expansion with multiple cysts, and third-space effusion of fluid, renal failure, hypovolemic shock and respiratory distress syndrome. Extracts of the fruits of Vitex agnus castus (VAC) have dopaminergic and anti-angiogenesis activities.
Objective: In this study, we sought to evaluate the effect of hydro-alcoholic extract of VAC on OHSS complication in a rat model.
Materials and Methods: OHSS was induced through the intra-peritoneal injection of pregnant mare serum gonadotrophn (PMSG) and treated groups were administered with 25 mg/kg of VAC extract. Body and ovary weights were measured. Vascular permeability (VP) was determined through the injection of 2 mL of 5 mM Evans Blue and staining was quantified at 600 nm using a spectrophotometry. Gene expression of the vascular endothelial growth factor (VEGF) and Pigment epithelium-derived factor (PEDF) were measured with semi-quantitative real-time PCR.
Results: The increase in body that observed in OHSS group were attenuated significantly by VAC extract application (p<0.05). The ovarian weight and diameter

were increased in OHSS group and treatment of rats with VAC extract diminished ovarian weight and diameter ($p<0.05$). Vascular permeability was significantly decreased in VAC treated rat in compared to control OHSS rats ($p<0.01$). VEGF gene expression was significantly increased and PEDF gene expression was decreased in the OHSS Group compared with the control groups ($p<0.05$). Both VEGF and PEDF gene expression alteration were significantly improved in VAC treated rats.

Conclusion: The results of the present study indicate that VAC extract can improve OHSS complication by reducing ovarian diameter, VP, and VEGF expression and increased PEDF expression in a rat model.

Key words: *OHSS, Rat Model, Vitex agnus, VEGF, PEDF.*

P-107

Review of human rights, legal, ethical and religious aspects of surrogacy in Iran with an emphasis on its counseling issues

Jahani Shourab N, Latifnejad Roudsari R.
Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: jahanishn@mums.ac.ir

Background: The participation of a third person in the process of infertility treatment will have various human rights, legal, ethical and religious aspects.

Objective: The present review, therefore, designed to examine the human rights, legal, ethical, and religious aspects of surrogacy in Iran, with an emphasis on its counseling issues.

Materials and Methods: The present study reviews the findings of published papers regarding different aspects of surrogacy in Iran between 2006 and 2016. Various databases including Medline, Pub Med, CINAHL, Google Scholar, and Iranian databases of SID and Iran Medex were searched using key words of the "surrogacy", "surrogate mother", "Third party reproduction", "human rights", "law", "ethics" and "religion". Eventually, 42 retrieved articles out of 706 were selected in the review.

Results: Although surrogacy has been accepted in Iranian law, but different aspects is not clear yet. There are still differences among Shiite and Sunni scholar's´ viewpoints. These whole issues increase the importance of comprehensive and continuous counseling of couples who seek this procedure as a therapeutic approach for their infertility.

Conclusion: One of the strategies to solve this issue is through developing comprehensive and accessible guidelines by infertility clinics, and follow up this problems.

Key words: *Surrogacy, Human rights, Ethics, Religion, Counseling.*

P-108

Role of imaging tools in breast screening of women undergoing ART treatment: All things that a midwife should know

Javam M, Ahmadi F.
Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: maryam_javam@yahoo.com

Background: This article is aimed to introduce the role of imaging tools for midwives in breast screening of women undergoing ART treatment.

Materials and Methods: A narrative review was performed on articles published at PubMed, Elsevier, Google scholar, SID, etc. and textbooks of infertility and imaging, using following keywords: breast cancer, ultrasonography, mammography, MRI. Several normal and abnormal mammograms, ultrasound and MRI images are provided to introduce normal and abnormal findings to midwives.

Results: Breast cancer is the most common cancer occurred in women. Those women who undergo IVF/ICSI are at higher risk of breast cancer, due to high level of sexual hormones injected during treatment cycles. Therefore, it's vital to screen these high-risk women. Several imaging tools are used to assess women of reproductive age to early diagnose suspicious lesions, including ultrasonography, mammography and MRI. Each method has its own pros and cons. Since many midwives work at infertility treatment clinics, they need to learn about the role of these imaging tools in breast screening of women undergoing ART treatment and abnormal findings reported by radiologists. These subjects are provided in this manuscript.

Conclusion: Midwives working at infertility treatment clinics should learn about imaging tools used for breast screening and abnormal findings reported by radiologists.

Key words: *Breast cancer, Ultrasonography, Mammography, MRI.*

P-109

Eugenol recovers some endocrine and histological changes in a rat model of polycystic ovary syndrome

Kokabian Z[1], Yaghmaei P[1], Hajebrahimi Z[2].
1.*Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran.*
2.*Aerospace Research Institute, Ministry of Science Research and Technology, Tehran, Iran.*
Email: yaghmaei_p@srbiau.ac.ir

Background: Polycystic ovary syndrome or PCOS is one of the most common cases of infertility among women of reproductive age. It is an endocrine disorder with inflammatory aspects leading to lack of ovulation. Eugenol is an active phenolic constituent of clove essential oil with the anti-inflammatory properties.

Objective: The aim of this study was to evaluate the therapeutic effects of eugenol in rats suffering from PCOS

Materials and Methods: 32 adult Wistar rats were divided into four groups of control, PCOS, PCOS + intra peritoneal injection of eugenol (12 and 24 mg/kg) for 30

days. The induction of syndrome was done by intra peritoneal injection of 4 mg of Estradiol valerate for 28 days. Control group received no injection. At the end of the trial, the serum and ovary of all groups were collected in order to investigate the histological and serological changes. One-Way ANOVA (one way analysis of variance) with Tukey test was used for analyzing the data and comparison of the different group means.
Results: Administration of eugenol decreased the serum level of estradiol and LH hormone and increased the serum level of FSH in treated rats when compared with PCOS rats ($p<0.05$). A significant increase was observed in the number of corpus luteum in the eugenol-treated groups compared with PCOS group ($p<0.05$).
Conclusion: Our results suggest that eugenol has improvement effects on an ovarian function and ovulation in the PCOS rat model. Therefore, it could be use as a protective agent and as an adjunct treatment in PCOS patients.
Key words: *Polycystic Ovary Syndrome, Eugenol, Rat.*

P-110
Predictors of teenage motherhood

Mangeli M.
School of Nursing and Midwifery, Islamic Azad University, Shahrbabak Branch, Kerman, Iran.
Email: m.mangeli@shahrbabakiau.ac.ir

Objective: Teenage motherhood is one of the important health challenges in developing countries. According to the WHO estimates, 10% of all worldwide births are delivered by adolescent mothers. Teenage motherhood impacts on mothers, children, families and communities. Thus their causes and predisposing factors must be explored. The aim of this study was to explore the factors that predict teenage motherhood in Iranian culture.
Materials and Methods: Inductive Conventional content analysis approach was used in this qualitative study. Face to face In-depth semi-structured interviews were conducted with 18 Iranian adolescent mothers in the Kerman province of Iran. Data collection continued until the point of data saturation and MAXQDA software (v. 10.0) was utilized in the analysis of the data.
Results: Two main categories (external motivators and internal motivators) and 9 sub-categories (inappropriate economic condition, breakdown of family, encouragement of parents, copying others, country policy; position, subjective beliefs, inner needs and desires, knowledge deficit) were extracted from the data.
Conclusion: The finding of this study showed that various factors (personal, social, economic, cultural, religious and technological) predict teenage motherhood. In order to overcome this problem and to address the associated issues; the adolescents, family, schools, society, religious centers, researchers, health centers, and policy makers must be considered. Understanding causes of teenage motherhood help health care providers to provide appropriate assessment and interventions for improve the health of teenage mothers. Health care providers can introduce adolescents to support centers; and providing appropriate care, counseling and education for them and their parents. Adolescent friendly reproductive health services are necessary for help to adolescent mothers.
Key words: *Adolescent mothers, Predictors, Iran, Qualitative, Kerman.*

P-111
Consequences of Teenage Motherhood

Mangeli M.
School of Nursing and Midwifery, Islamic Azad University, Shahrbabak Branch, Kerman, Iran.
Email: m.mangeli@shahrbabakiau.ac.ir

Background: Teenage motherhood is a major health challenge in most developing countries. World Health Organization reported that each year, sixteen million adolescents become mother. Early motherhood in adolescent is associated with consequences in health status, education, and socioeconomic condition.
Objective: The aim of this study was to explore the consequences of teenage motherhood in Iranian cultures.
Materials and Methods: This qualitative study was done using the conventional inductive content analysis approach. A purposive sample of eighteen Iranian mothers, with the experience of early motherhood, was recruited with maximum variation in terms of their age at their first pregnancy, their children's age, place of residence, and financial status. Data collection was done via in-depth semi-structured interviews and continued up to data saturation. The MAXQDA software (v. 10.0) was employed for handling the data.
Results: Iranian mothers' experiences of the outcomes of teenage motherhood extracted into two main categories which were named "positive outcomes of early motherhood" and "negative outcomes of early motherhood". The four subcategories of the first category were the acceleration of intellectual and mental maturation, strengthening of family relationships, developing a strong identity, and closer companionship with the child. The second main category also included four subcategories, namely experiencing numerous difficulties, threats to mothers' physical and mental health, threats to children's health, and missing opportunities.
Conclusion: Teenage motherhood is not merely a negative experience; rather it is also associated with different positive outcomes. Healthcare providers, particularly nurses, need to provide high quality prenatal, perinatal, and postnatal care services to adolescent mothers and use available opportunities to strongly support them and their children.
Key words: *Adolescent mothers, consequences, Qualitative, Iran.*

P-112
Comparison of pregnancy outcomes in patients undergoing frozen embryo transfers with or without depot gonadotropin-releasing hormone agonist pretreatment

Mehrafza M, Zare Yousefi T, Hosseinzadeh E, Raoufi A, Samadnia S, Aghajani S, Hosseini A.
Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran.
Email: marzieh.mehrafza@gmail.com

Background: Embryo cryopreservation is a safe and highly effective method to improve assisted reproductive technique (ART) outcomes. Protocols for endometrial preparation in frozen-thawed embryo transfer (FET) cycles include natural cycles and hormonal replacement cycles with or without gonadotropin-releasing hormone (GnRH) agonist. The superiority of one method over others remains controversial.
Objective: The aim of the present study was to compare pregnancy outcomes between patients undergoing artificial endometrial preparation with or without GnRH agonist.
Materials and Methods: One hundred ninety six patients undergoing artificial endometrial preparation for FET were retrospectively evaluated in the present study. According to pretreatment with GnRH agonist, patients were divided into 2 groups: patients who received half dose of GnRH agonist (decapeptyle, 1.87 mg) on day 21 of the preceding cycle (group A, n=93) versus who did not receive (group B, n=103).
Results: Baseline characteristics of patients were not different between two groups. There was no significant different in endometrial thickness (p=0.92), total number of embryos transferred (p=0.86), number of blastocyst transferred (p=0.57) and number of grade A embryos transferred (p=0.64) between 2 groups. No significant differences were seen in implantation (15% vs. 14%, p=0.76), fertilization (74% vs. 74%, p=0.72), biochemical (36.6% vs. 34%, p=0.77) and clinical pregnancy rate (29% vs. 30.1%, p=0.87) between two groups.
Conclusion: The results of present study indicated that artificial endometrial preparation without GnRH agonist seems to be as efficient as with GnRH agonist.
Key words: *Embryo transfer, Gonadotropin-Releasing Hormone, Pregnancy, Cryopreservation.*

P-113
Assisted reproductive outcomes in women with different polycystic ovary syndrome phenotypes

Mirhashemi ES.
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: eeam3663@gmail.com

Background: With an incidence of 5-8% worldwide, polycystic ovary syndrome (PCOS) is considered one of the most prevalent endocrinopathies in women of reproductive age.
Objective: To explore assisted reproductive outcomes among women with different polycystic ovary syndrome (PCOS) phenotypes.
Materials and Methods: A retrospective cohort study included women with PCOS who were treated at Yazd Research and Clinical Center for Infertility, Yazd, Iran, using the GnRH antagonist protocol between April 1, 2015, and August 31, 2017. Clinical pregnancy was the primary outcome, and chemical pregnancy, implantation rate, fertilization rate, and spontaneous abortion rate, were the secondary outcomes that were evaluated among four defined phenotypes of women with PCOS.
Results: Significant differences were observed between the phenotypes for the levels of luteinizing hormone, anti-Müllerian hormone, fasting blood sugar, and total testosterone concentration; similarly, the percentage of women with luteinizing hormone/ follicle stimulating hormone ratio of at least 2.5 differed between PCOS phenotypes (all $p<0.001$). There were also significant differences in estradiol level ($p<0.001$) and the number of matured follicles ($p=0.002$) between different phenotypes. No significant differences were observed in the fertilization and implantation rates, as well as chemical and clinical pregnancy rates (all $p>0.05$).
Conclusion: No significant differences were observed in assisted reproductive technique outcome among women with different phenotypes of PCOS undergoing frozen-thawed embryo transfer.
Key words: *Assisted reproductive technology, Fertilization rate, Implantation rate, Ovarian hyperstimulation, PCOS, PCOS phenotypes, Pregnancy rate, Spontaneous abortion rate.*

P-114
An epidemiologic survey on the causes of infertility in patients referred to the Majesty of Maryam Infertility Center, Beheshti Hospital, Isfahan

Mohammadi P[1, 2, 3], Bagheri N[1], Eshaghi Sh[1], Torkiyan N[1], Fadaee M[1], Naghshineh E[1, 4].
1. *Majesty of Maryam Infertility Center, Martyr Beheshti Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
3. *Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
4. *Department of Obstetrics and Gynecology, Infertility Center, Beheshti University Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.*
Email: pmanatomist@gmail.com

Background: Infertility is considered as a major health care problem of different communities. The high prevalence of this issue doubled its importance. A significant proportion of infertility have been related to environmental conditions and also acquired risk factors. Different environmental conditions emphasized the need to study the different causes of infertility in each area.
Objective: The aim of this study was to determine the frequency causes of infertility in infertile couples.
Materials and Methods: In this cross sectional descriptive study 3603 infertile men and women that were referred to infertility clinic of Maryam infertility center in Beheshti Hospital during 2017 to 2018, were examined. This center is the only governmental center for infertility in Isfahan. Sampling was based on census method. Information about the patients was obtained from medical examinations and laboratory findings. To

analyze the data, descriptive statistics such as frequencies and the mean were used.

Results: The prevalence of primary and secondary infertility was 71.43% and 28.57% respectively. The etiology of infertility in couples revealed; male factor in 22.2%, female factor in 50%, combined factors in 4.17% and undetermined cause in 23.61%. In the causes of female infertility, menstrual disorders, diseases (obesity, thyroid diseases, and diabetes), ovulation dysfunction, uterine factor, fallopian tubes and cervical factor had the highest prevalence respectively. The causes of male infertility based on their frequency included semen fluid abnormalities, genetic factors, vascular abnormalities, and anti-spermatogenesis factors, respectively.

Conclusion: Etiology pattern of infertility in our study is similar with the many other patterns that have been reported by the World Health Organization. This study showed that in central part of Iran (Isfahan province), female factor as the main cause of infertility (50%). Therefore, there is a need to revise public health program on infertility to focus on the education and prevention of infertility and its risk factors.

Key words: *Male infertility, Female infertility, Etiology, Epidemiology.*

P-115

Association between metabolic syndrome and polycystic ovary syndrome among adolescents: A review article

Moradi F[1], Ghadiri-Anari A[2], Enjezab B[3].

1.*Student Research Committee, Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Internal Medicine, Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Midwifery, Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences Yazd, Iran.*

Email: enjezabbehnaz@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is a complex reproductive endocrinology disease, affecting 5-20% of both the adolescent and reproductive women. This syndrome has significant clinical consequences associated with reproductive, metabolic and psychological problems. Some PCOS and metabolic syndrome female phenotypes share common characteristics. Metabolic syndrome has increased worldwide. The current epidemic of childhood and adolescence obesity may increase the risk of PCOS and metabolic syndrome and its complications in lower ages.

Objective: The aim of this review article was study of association between metabolic syndrome and polycystic ovary syndrome among adolescents.

Materials and Methods: For this purpose, 54 initial articles obtained and 34 articles from 2006 to 2018 were reviewed with the keywords of polycystic ovary syndrome, metabolic syndrome, adolescents and childhood obesity in PubMed, Google Scholar, Science Direct and Springer Link.

Results: In the majority of studies, method was descriptive-correlational and analytical-cohort study and 7 review articles. These papers had explained about characteristics of polycystic ovary syndrome in adolescents and its relationship between metabolic syndrome, that according to, Body mass index at adrenarche is an important risk factor for development of insulin resistance in pubertal ages. Hormonal fluctuations which lead to metabolic changes during transition to adolescence may mimic the features of metabolic syndrome. Adolescents with PCOS are at higher risk for comorbidities such as metabolic syndrome and its components. Although many studies have shown central obesity is associated with hyperandrogenism, results of some studies indicate that hyperandrogenism and its associated parameters are significantly associated with metabolic syndrome in non-obese PCOS patients, whereas this association was not observed in obese patients. So hyperandrogenaemia is a risk factor for metabolic syndrome independent of obesity and insulin resistance. Unlike obese adults, PCOS and its individual components were not associated with metabolic syndrome in the untreated morbidly obese adolescent population.

Conclusion: It seems that PCOS phenotypes cannot be used to predict metabolic disorders, so all adolescents with PCOS should be fully evaluated to determine baseline metabolic parameters on their first visit independently of their BMI.

Key words: *Polycystic ovary syndrome, Metabolic syndrome, Adolescents, Childhood obesity.*

P-116

The effectiveness of lifestyle modification on women with polycystic ovary syndrome: A scientific review

Moradi F[1], Enjezab B[2].

1.*Student Research Committee, Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Midwifery, Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: enjezabbehnaz@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder with an estimated prevalence of 4-25% depending on diagnostic criteria. This syndrome has significant and diverse clinical consequences associated with reproductive problems (menses disorders, failure to ovulate, late menopause, endometrial cancer and infertility), metabolic problems (insulin resistance, diabetes type 2, dyslipidemia, hypertension, and cardiovascular diseases), physical problems (central obesity, acne, hirsutism and hair loss) and psychological problems (depression, stress and anxiety). Obesity, as one of the most important symptoms of this syndrome, alone is a risk factor for many diseases that occurs in more than 50% of women with polycystic ovary syndrome. Lifestyle is combination

of behavioral patterns and habits; and various factors affect lifestyle of obese women.
Objective: The aim of this study was review of effect of lifestyle modification on women with polycystic ovary syndrome.
Materials and Methods: 47 related articles were reviewed from 2010 to 2018 with the keywords of polycystic ovary syndrome, lifestyle, diet, physical activity and sleep quality from resources: PubMed, Google Scholar, Science Direct, Wiley Online Library and Springer.
Results: In the majority of studies, method was interventional- clinical trials and also 12 review articles were considered. All papers had examined the effect of healthy lifestyle on polycystic ovary syndrome, that according to, Physical activity through weight loss and reduction in body mass index have beneficial effects on metabolic, hormonal, ovarian and menstrual parameters, moreover; physical activity improve mental health and throughout improve quality of life in these patients. Also, dietary regeneration through caloric restriction affect improving reproductive and metabolic disturbances. Adequate sleep in suitable condition is helpful in improving the quality of life of these patients through adjusting the levels of neurohormones.
Conclusion: Due to the positive effect of lifestyle modification as an inexpensive and non-invasive method, counseling based on lifestyle modification along with medical management is recommended to patients with polycystic ovary syndrome.
Key words: *Polycystic ovary syndrome, Lifestyle, Diet, Physical activity, Sleep quality.*

P-117
Levothyroxine treatment and pregnancy outcome in women with subclinical hypothyroidism: A systematic review and meta-analysis

Nazarpour S[1, 2], Ramezani Tehrani F[2], Amiri M[2], Bidhendi Yarandi R[3], Azizi F[2].
1.*Department of Midwifery, Varamin-Pishva Branch, Islamic Azad University, Tehran, Iran.*
2.*Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3.*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
Email: ramezani@endocrine.ac.ir

Background: Subclinical hypothyroidism (SCH) is the most common thyroid dysfunction during pregnancy. Despite well-defined clinical guidelines on treatment of overt hypothyroid in pregnant women, there is no consensus on Levothyroxine (LT4) treatment of SCH women.
Objective: In this meta-analysis we aimed to evaluate the benefits of Levothyroxine treatment on pregnancy outcomes in SCH women.
Materials and Methods: PubMed [including Medline], Web of Science, Wiley, Google Scholar, Science direct and Scopus were searched for retrieving articles published in English language on effects of Levothyroxine treatment on pregnancy outcomes in pregnant women with SCH compared to untreated or healthy controls up to 2018. In this systematic and meta-analysis, both fixed and random effect models were applied to estimate pooled effect size. Heterogeneity and publication bias were evaluated using the I-squared (I^2) and Begg's statistics, respectively.
Results: Twelve cohort studies and randomized controlled trials with a total of 11307 participants were analyzed. This meta-analysis showed that pregnant women with SCH treated with levothyroxine had lower chances for pregnancy loss (OR: 0.71, 95% CI: 0.57-0.88; I^2=0%) and higher chances for live birth rate (OR: 2.72, 95% CI: 1.44-5.11; I^2=25%) than placebo group. Compared to euthyroid women, patients with SCH treated with levothyroxine had a higher odds ratio for preterm labor (OR: 1.80, 95% CI: 1.13-2.86; I^2=0%). There was not any significant difference in chances of other adverse maternal complications, obstetrical hemorrhage, and neonatal/NICU admission between patients with SCH treated with levothyroxine and control groups (placebo and euthyroid).
Conclusion: This study shows that the effect of treatment with levothyroxine in pregnant women with SCH is not the same for all pregnancy outcomes. Levothyroxine treatment in these patients can reduce the loss of pregnancy. Due to the limited number of studies, further studies are needed to conclude more precisely about other consequences.
Key words: *Subclinical hypothyroidism, Pregnancy, Outcome, Treatment.*

P-118
The effect of vitamin D supplementation on the androgenic profile in atients with olycystic ovary syndrome: A systematic review and meta-analysis of clinical trials

Azadi-Yazdi M[1, 2], Nadjarzadeh A[1, 2], Khosravi-Boroujeni H[3, 4], Salehi-Abargouei A[1, 2].
1.*Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Menzies Health Institute, Queensland, Australia.*
4.*School of Medicine, Griffith University, Queensland, Australia.*
Email: abargouei@ssu.ac.ir

Background: It is suggested that vitamin D status is associated with androgenic profile in women with polycystic ovarian syndrome (PCOS). Although several clinical trials are known in this regard, the results were inconsistent.
Objective: Therefore, this study was aimed to conduct a systematic review and meta-analysis of published clinical trials to elucidate the possible effect of vitamin D supplementation on the androgen levels in adult females with PCOS**.**
Materials and Methods: PubMed, SCOPUS, and Google Scholar were searched to identify related articles

published up to January 2017. Mean ± standard deviation (SD) of changes in serum total testosterone, sex hormone binding globulin (SHBG), and free testosterone were extracted to calculate Hedges' g to be used as effect size for meta-analysis. DerSimonian and Liard random effects model was incorporated to summarize the effects. Six clinical trials with 183 participants aged 18-41 yr with follow-up period between 3-24 wk were included**.**

Results: Our analysis revealed that vitamin D supplementation significantly reduces total testosterone (Hedges' g=-0.32, 95% CI: -0.55 to -0.10; p=0.005); this effect remained significant in single group trials after subgroup analysis. Vitamin D supplementation did not affect serum free testosterone (Hedges' g=-0.21, 95% CI: -0.44 to 0.079; p=0.08) or SHBG levels (Hedges' g=0, 95% CI, 0.22-0.22; p=0.98).

Conclusion: The present systematic review and meta-analysis revealed that vitamin D supplementation might significantly affect serum total testosterone while it is not effective in improving other markers of androgenic profile. Future double-blind, placebo-controlled clinical trials are highly recommended.

Key words: *Vitamin D, Androgens, Polycystic ovary syndrome, Systematic review, Meta-analysis.*

P-119

The relationship between hyperlipidemia and maternal and neonatal outcomes in pregnant women admitted to Al-Zahra Hospital in Rasht

Sharami SH, Alizadeh F.

Department of Obstetrics and Gynecology, Reproductive Health Research Center, Guilan University of Medical Sciences, Rasht, Iran.

Email: sharami@gums.ac.ir

Background: Concentrations of plasma lipids levels during pregnancy clearly increases. According to some studies, dyslipidemia is effective in the incidence of preeclampsia and insulin resistance.

Objective: The present study aimed to examine the relationship between hyperlipidemia and maternal and neonatal outcomes in pregnant women admitted to Al-Zahra Hospital in Rasht.

Materials and Methods: In this cross-sectional study, women were divided into two groups of pregnant mothers with hyperlipidemia and normal ones. Data including gestational age at birth, maternal age, maternal BMI, maternal weight gain during pregnancy, gestational diabetes mellitus, preeclampsia, cholestasis, delivery method and neonatal outcomes including birth weight and Apgar score were gathered. Data were analyzed by Chi-square, independent T-test, and Mann- Whitney U test in SPSS 21. To compare the relationship between hyperlipidemia and maternal and neonatal outcomes with controls, the logistic regression test and the Odds ratio were used. P<0.05 indicated statistical significance.

Results: Results showed that, the higher the gestational age, the greater the incidence of abnormal lipid parameters. In pregnant women with dyslipidemia in combination with increased TG, CHOL and LDL, and decreased HDL, the incidence rates of gestational diabetes, preeclampsia, cholestasis, FGR, and macrosomia were statistically higher (p<0.05) and controlling dyslipidemia could prevent these disorders.

Conclusion: This study suggested that dyslipidemia was associated with some adverse effects of pregnancy and harmful fetal outcomes. Therefore, it seems that adding laboratory assessment of lipid profiles before and during pregnancy can be effective in early diagnosis of dyslipidemia.

Key words: *Dyslipidemias, Gestational diabetes, Pre-Eclampsia, Fetal macrosomia.*

P-120

The relation between free testosterone and components of metabolic syndrome in women with polycystic ovary syndrome

Sharami SH, Dalil Heirati S.

Department of Obstetrics and Gynecology, Reproductive Health Research Center, Guilan University of Medical Sciences, Rasht, Iran.

Email: sharami@gums.ac.ir

Background: Previous investigation demonstrated 5 fold increased risk of metabolic syndrome (MS) in patients at the highest quartile of free testosterone level but not for total testosterone. They recommended further studies to investigate the role of hyperinsulinemia as an interface for hyperandrogenism and MS**.**

Objective: The aim of this study is to assess the relationship between free testosterone level and components of MS in women with PCOS.

Materials and Methods: This is a cross-sectional study which was conducted on 215 women with PCOS. PCOS was diagnosed based on the Rotterdam criteria. Patients were divided into two subgroups of patients with and without MS based on ATP III criteria. In each subgroup, the association between individual components of MS with free testosterone was measured. Data were analyzed using SPSS software.

Results: The prevalence of MS was 28.8% (n=62). The mean level of free testosterone in patients with blood pressure ≥130/85 was significantly higher than those with blood pressure <130/85 mm/hg. (p=0.029) Also, in patients with diastolic blood pressure ≥85, the level of free testosterone was significantly higher than patients with diastolic blood pressure <85 (p=0.026). Results showed significant positive correlation between the level of free testosterone and cholesterol (p=0.024). But no significant correlation was noted between levels of free testosterone and other variables.

Conclusion: Regarding the relationship between blood pressure and high levels of free testosterone, it seems that regular blood pressure screening has a higher priority of concern comparing other complications for preventing cardiovascular adverse effects in women with PCOS and hyperandrogenism.

Key words: *Testosterone, Polycystic ovary syndrome, Metabolic syndrome, Hyperandrogenism.*

P-121
Comparison of free Beta-HCG and PAPPA in single normal and under IVF pregnancy at 12 weeks of gestational age

Talayeh M, Taheripanah R.
Department of Obstetrics and Gynecology, Infertility and Reproductive Health Research Center (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: m_talayeh@yahoo.com

Background: In some previous studies first trimester screening tests results are equivocal in IVF pregnancies and true interpretation is crucial.
Objective: The purpose of this study was to compare Free Beta-HCG and PAPPA levels in single normal and under IVF pregnancies at 11-13w+6d of gestational age.
Materials and Methods: In this observational study that was performed as a cohort, 300 consecutive single IVF pregnancies and 700 single normal pregnancies were enrolled at about 11-13w+6d of gestational age and Free Beta-HCG and PAPPA was compared across the groups.
Results: The results in this study demonstrated that PAPPA (p=0.026) was significantly lower and beta-HCG (p=0.030) was significantly higher in IVF pregnancies. The other factors including NT (Nuchal Translucency) and CRL (crown to rump length) and demographic characteristics were not significantly differed across the groups (p>0.05).
Conclusion: This study showed that, PAPPA is lower and Free Beta-HCG is higher in single IVF versus normal pregnancies. It could be related to different placentation in ICSI technique because of alteration in oocyte cytoplasm. Therefore, these markers may be needed to be adjusted in ART conceptions. Further research should be done to obtain optimal cut-off for these markers in first trimester screening for Down syndrome in ART pregnancies.
Key words: *Free Beta-HCG, PAPPA, IVF pregnancy.*

P-122
Survey of fertility motivations and some related factors in women of reproductive age who referred to health centers in Sabzevar in 2016

Zare Z[1], Laal Ahangar M[2], Kiaee Tabar R[3].
1.*Department of Midwifery, Neyshabor University of Medical Sciences, Neyshabur, Iran.*
2.*Department of Nursing, Sabzevar University of Medical Sciences, Sabzevar, Iran.*
3.*Department of Midwifery, Sabzevar University of Medical Sciences, Sabzevar, Iran.*
Email: zare1984@yahoo.com

Background: Considering the severe decline in fertility rates in Iran, which could cause irreparable economic and social damage to the country, it is necessary to survey the effective factors on the reduction of fertility rates.
Objective: The purpose of this study was to investigate the fertility motivations and some related factors in women of reproductive age who referred to health centers in Sabzevar in 2016.
Materials and Methods: This cross-sectional study was performed on 450 women (in age 18-35 years) with method of clustered. Data collection tools included demographic questionnaire, Miller's Childbearing Questionnaire. Data were analyzed using descriptive statistics, Pearson correlation tests, Spearman, and Kruskal Wallis tests. The significance level was p<0.05.
Results: The average score of positive fertility motivation was 22.64±0.33 and the negative motives was 17.1±3.85. There was a significant and reverse correlation between positive fertility motivation with Education level (p=0.01) and between the negative fertility motivation with income level (p=0.001). There was a significant and direct correlation between the positive fertility motivation with age (p=0.01), number of pregnancies, childbirth and children (p=0.001).There was a significant relationship between child gender, lodging and spouse's occupation (p=0.01).
Conclusion: The results of this study showed that age, education level, number of children and pregnancies and delivery, income level, spouse's occupation, place of lodging, having both of child sex, duration of marriage and female employment are factors related to fertility motives.
Key words: *Fertility motivations, Women, Fertility, Related factors.*

5- Psychology

P-123
From physical to financial infertility: A grounded exploration of consequences of infertility among infertile female

Abooei A[1], Afshani S[2], Fallah M[1], Rouhani A[2].
1.*Department of Psychology, Islamic Azad University, Yazd Branch, Yazd, Iran.*
2.*Department of Sociology, Yazd University, Yazd, Iran.*
Email: azabooei@gmail.com

Background: The infertility is a stressful event in the human life and is now considered as a relatively common problem among couples. According to world statistics, a couple out of every eight couples is struggling with the infertility.
Objective: The present study aimed to investigate physical and financial aspects of infertility in infertile female referring to Yazd Reproductive Sciences Institute.
Materials and Methods: 21 females were selected and studied using a qualitative approach, the grounded theory, and the theoretical sampling. The sampling process continued until the data saturation. Data was collected and analyzed using the open and axial coding.
Results: The research findings included 14 main categories namely high medical expenses; travel expenses; lack of facilities; stress and financial concerns; strategies for attracting financial resources; inequality of income and expenses; treatment abandon; side effects of drugs; negative effects on the hair growth; skin complications; drug inefficacy; voracious appetite; body

vulnerability; and negative effects of aging as well as a core category called expenses of parenting. Conceptual and paradigmatic tables were consequently presented.
Conclusion: The explanation of results generally indicated physical and financial problems in the target population leading to a double burden on them.
***Key words**: Infertile female, Physical problems, Financial problems, Infertility.*

P-124
Associations between clinical and biochemical hyperandrogenism and quality of life in patients with PCOS

Amiri M, Ramezani Tehrani F, Bidhendi Yarandi R.
Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: ramezani@endocrine.ac.ir

Background: Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders, which is characterized by chronic oligo-ovulation or anovulation and hyperandrogenism (HA) resulting in menstrual irregularities, infertility, hirsutism, acne, and alopecia; these manifestations can lead to psychosocial and emotional disturbances and a decrease in quality of life (QoL) of PCOS patients.
Objective: To evaluate the association between clinical and biochemical parameters of HA and quality of life in PCOS patients.
Materials and Methods: In this cross-sectional study, we assessed the associations between clinical and biochemical parameters of HA and different aspects of QoL in a total of 211 women with PCOS using a specific health related quality of life questionnaire designed for PCOS patients.
Results: This study showed a negative association between FAI and total QoL (p=0.042) and domains of hirsutism (p=0.045) and obesity-menstrual (p=0.001). Dehydroepiandrosterone sulfate (DHEAS) was positively associated with the sexual function aspect of QoL (p=0.043). There were no significant associations between QoL and other hormonal parameters including luteinizing hormone (LH) to follicle stimulating hormone (FSH) ratio and total testosterone (tT).
Conclusion: Our study demonstrated the destructive impact of PCOS clinical and biochemical HA on several aspects of the patients' quality of life. Although biochemical disturbances can influence QoL of patients with PCOS, our results highlighted the role of clinical signs of PCOS such as obesity, infertility and hirsutism in deteriorating QoL. Hence, clinicians should regularly assess the clinical and psychosocial dimensions of PCOS as well as biochemical aspects.
***Key words:** Quality of life, Hyperandrogenism, Infertility, Obesity, Polycystic ovary syndrome.*

P-125
Sexuality and infertility

Bokaie M[1], Yassini SM[2].
1. *Department of Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Psychiatric, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: mah_bokaie@yahoo.com

Background: Sometimes some sexual problems lead to infertility while in some cases infertility caused sexual problem. Cause of infertility, length of infertility and family and social support influence the couples sexual life.
Objective: The aim of this study was to show how sexuality change after diagnose of infertility.
Materials and Methods: In this review article we survey 176 articles from 2000-2017 and analyzed 36 related articles in pubmed, scopus, science direct and Iranian database.
Results: We found most changes in sexual self-esteem, sexual relationship, sexual function, marital adjustment, psychological change such as anger, panic, despair, and grief and times of sexual activity.
Conclusion: One of the aims of infertility sexual counseling is to help the couples to separate sex from reproduction. We believe sexual counseling in infertility center is very helpful and improve sexual health of infertile couples.
***Key words:** Sexuality, Infertility, Sexual counseling, Sexual function, Infertile couple.*

P-126
Infertile couples' needs after unsuccessful fertility treatment: A qualitative study

Ebrahimzadeh Zagami S[1], Latifnejad Roudsari R[2], Janghorban R[3], Mousavi Bazaz S[4], Amirian M[5], Helen TA[6].
1. *Department of Midwifery, Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Midwifery, Research Center for Patient Safety, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *Department of Midwifery, Community Based Psychiatric Care Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
4. *Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
5. *Department of Obstetrics and Gynecology School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
6. *Department of Adult Child and Midwifery School of Health and Education, Middlesex University, London, UK.*
Email: latifnejadr@mums.ac.ir

Background: Infertility is a major medical issue. Investigations and treatment are the beginning of a complex, time-consuming and stressful process for couples that may well fail.
Objective: The present study explored the needs of infertile couples following treatment failure with Assisted Reproductive Technologies.
Materials and Methods: A descriptive qualitative study was conducted in an Iranian infertility center, in the Northeast of the country between April 2016 and June 2017. Researchers recruited 29 individuals including 9

couples, 9 women and two men with primary infertility through purposive sampling. Data were collected using semi-structured interviews and analyzed iteratively using conventional content analysis with MAXQDA software.
Results: The main concepts obtained from the data were classified into four main categories along with their subcategories, and included the need for psychological support, the need for more useful information, the need for social support and the need to access to better services.
Conclusion: The findings show that following treatment failure, these infertile patients' expressed needs and preferences were not met. Identifying and meeting their needs may help infertile couples to deal with IVF failure and to reach a decision about future treatment.
Key words: *Infertility, Fertility treatment, Assisted reproductive technology, Need, Qualitative study.*

P-127
A qualitative study of the experiences of Iranian infertile couples after unsuccessful assisted reproductive technologies

Ebrahimzadeh Zagami S[1], Latifnejad Roudsari R[2], Janghorban R[3], Mousavi Bazaz S[4], Amirian M[5], Helen TA[6].
1.*Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
2.*Research Center for Patient Safety, Mashhad University of Medical Sciences, Mashhad, Iran.*
3.*Community Based Psychiatric Care Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
4.*Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
5.*Department of Obstetrics and Gynecology School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
6.*Department of Adult Child and Midwifery School of Health and Education, Middlesex University, London, UK.*
Email: latifnejadr@mums.ac.ir

Background: Assisted reproductive technologies (ARTs) give hope to some infertile couples; however In Vitro Fertilization (IVF) is expensive and not subsidized by the Iranian state.
Objective: The aim of this paper is to describe the experiences of Iranian infertile couples after unsuccessful treatment.
Materials and Methods: In a descriptive qualitative study 36 participants including 29 Iranian infertile couples recruited after unsuccessful ARTs treatment, five infertility treatment team members and two relatives of infertile couples, were interviewed at an Infertility Center in Northeastern Iran from April 2016 to June 2017. Data were collected using semi-structured, face-to-face interviews. Data analysis was carried out following Sandelowski (2000).
Results: Iranian infertile couples' experiences following failed ART cycles are described. The findings presented here show that Iranian infertile couples experience stressors during treatment cycles and systemic challenges which may be unique to the Iranian cultural context.
Conclusion: Iranian infertile couples face particular challenges related to the cultural context in which ARTs are delivered. Further exploration of the effects of culture on the experiences of failed ARTs need to be considered by infertility clinics in Iran.
Key words: *Assisted reproductive technologies, Infertile couples, Failed treatment cycles, Qualitative content analysis, Support.*

P-128
Infertile couples decision for re-treatment after unsuccessful treatment with assisted reproductive techniques

Ebrahimzadeh Zagami S[1], Latifnejad Roudsari R[2], Janghorban R[3], Mousavi Bazaz S[4], Amirian M[5].
1.*Department of Midwifery, Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
2.*Department of Midwifery, Research Center for Patient Safety, Mashhad University of Medical Sciences, Mashhad, Iran.*
3.*Department of Midwifery, Community Based Psychiatric Care Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
4.*Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
5.*Department of Obstetrics and Gynecology School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
Email: latifnejadr@mums.ac.ir

Background: The use of assisted reproductive techniques (ART) is increasing in the world. Although ART have extensively progressed, only 35% of couples have been pregnant with this methods. If treatment is not successful, couples will face new decisions such as tolerance or lack of tolerance for subsequent treatment cycles.
Objective: The aim of the study was to determine the decision of infertile couples for re-treatment after unsuccessful treatment with ART.
Materials and Methods: In this qualitative study, the participants were selected using purposive sampling method. The data were collected using 36 semi-structured deep interviews from April 2016 to June 2017 at an Infertility Center in Iran. All interviews were recorded, transcribed, and analyzed using conventional content analysis method with MAXQDA software.
Results: Two main themes of exposure to incentives (consisting of five categories of the impact of compensatory mechanisms, perfect support of the spouse, encouragement to continue treatment by relatives, interaction with counterparts and the existence of conditions hoping for the continuation of the treatment) as well as looking for a chance again (consisting of three categories of getting financial preparation for continued treatment, confusion of emotional fluctuations and doubting how to continue treatment) were emerged.
Conclusion: The results showed that infertile couples after unsuccessful treatment, were encouraged to re-treatment and looked for an again chance by different sources and forms. Therefore, it is necessary that a supportive program to be implemented by in the treatment centers by specialist therapists after the failed treatment.

Key words: *Infertile couples, Assisted reproductive techniques, Unsuccessful treatment, Qualitative study.*

P-129

Comparison of adaptation of pregnancy and prenatal self-evaluation in women with and without experience of infertility

Ebrahimzadeh Zagami S[1], Golmakani N[1], Kabirian M[1], Shaban Z[1], Khadem N[2], Talebi M[3].

1. *Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Obstetrics and Gynecology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *Department of Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

Email: golmakanin@mums.ac.ir

Background: Pregnancy is one of the most important periods of women's lives. Many women get pregnant using ART after many years, although, their individual and social problems will not finish. Infertility treatments have the destructive effects of physical, economic, and psycho-emotional. Clinical experiences known women's attitudes and behavior change about pregnancy after ART. Less attention has been paid to the comparison of women's attitudes with and without experience of infertility.

Objective: Aim of this study was comparison of pregnancy adaptation and prenatal self-evaluation in women with and without infertility experience.

Materials and Methods: In this cross sectional study, 250 pregnant women with entry criteria were chosen by convenience sampling in Montaserieh Infertility Research Center and Mashhad Health centers. Pregnant women completed adaptation of pregnancy questionnaire and Prenatal Self-Evaluation questionnaire. In this study, first group were pregnant women with infertility experience that get pregnant with ART and second group were pregnant women without infertility experience. Data analyzed using Mann- Whitney, t-test, X2 and Cross Calves by SPSS16.

Results: The average age of pregnant women was 34.3±5.5 years in first group and 23.13±3.05 years in second group that there was significantly statistical difference between two groups (p=0.05). The majority of women (83%) were urban and 40% had high school education. Also 77% of pregnant women were housewife. There was not significantly statistical difference in place of living, education and job between two groups (p>0.05). In first group, 83% had primary infertility and the majority of women (59%) get pregnant with IVF. Infertility factor were female factor (30.2%), male factor (30.9%), mixed (20.9%) and unknown (17.3%). Number of treatment was between 1-10 treatment cycles and average treatment duration was 42.1±39.1 months. Pregnancy adaptation was less in women with infertility experience rather than spontaneous pregnancy (p=0.044). Prenatal self-evaluation was not significant in two groups (p=0.57).

Conclusion: Assisted reproductive techniques create problems in women adaptation with pregnancy. So, successful infertility treatment with ART does not involve easy adaptation to the new life. It is necessary to perform women's counseling and care by the medical staff.

Key words: *Pregnancy, Infertility, Adaptation of pregnancy, Pregnant women, Prenatal self-evaluation.*

P-130

Sexual satisfaction in women with polycystic ovary syndrome: A review article

Enjezab B[1], Moradi F[2].

1. *Department of Midwifery, Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Student Research Committee, Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: enjezabbehnaz@yahoo.com

Background: Polycystic ovary syndrome is a complex endocrine and metabolic disorder with an estimated prevalence of 5-24% depending on diagnostic criteria. The symptoms of this syndrome are irregular menstruation, hirsutism, alopecia, obesity, infertility and acne. These symptoms changes body appearance and can lead to diminished feminine identity and also psychological stress in these patients. Female sexuality is a complex behavioral trait that encompasses both biological and psychological components. Since there are multiple factors that can impair the sexual function of these patients, it is essential to evaluate the main factors associated to this disorder.

Objective: The aim of the study was review of the effect of polycystic ovary syndrome on sexual satisfaction.

Materials and Methods: From 45 initial obtained articles, 23 articles were reviewed from 2007 to 2018 with the keywords of polycystic ovary syndrome, sexual satisfaction and sexual function from resources: PubMed, Google Scholar, Science Direct, Springer and Wiley Online Library.

Results: In the majority of studies, kind of method was descriptive cross-sectional and correlational study and only one study was review article. All papers had investigated sexual satisfaction in women with polycystic ovary syndrome with different symptoms of this syndrome, that according to, polycystic ovary syndrome women with infertility, irregular menstruation, severe hirsutism, high body mass index and alopecia had significantly more sexual dysfunction and mood problems, depression, anxiety and lack of self-confidence impair these women's sexual function. In addition, women with limited or no formal education had more sexual dysfunction. Acne, low moderate hirsutism and hyperandrogenism didn’t have any important influence on body image and self-esteem and, as a consequence, on sexual function. Some expressed no difference in sexual dysfunction among women without and with polycystic ovary syndrome. Higher level of testosterone in these

women had positive correlation with their sexual function. It is possible that a positive association between androgen levels and satisfying sexual functioning is masked by the negative effects of this syndrome phenotype on self-esteem. Thus, their mental health has to be analyzed more specifically and it has to be considered that sexual function impairment can be intensified as disease progresses or because of side effects of used medications.

Conclusion: It seems appropriate to screen all patients with polycystic ovary syndrome for sexual function with a simple short questionnaire and targeted interventions such as sexual consultation could be considered to help improve their quality of life along with other treatments.

Key words: *Polycystic ovary syndrome, Sexual satisfaction, Sexual function.*

P-131

The effect of preconception care education of health volunteers based on the Health Belief Model on knowledge and attitude of women

Moradi M[1], Fazeli N[1], Khadivzadeh T[1], Esmaily H[2].

1. *Department of Midwifery, Faculty of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Biostatistics, Faculty of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*

Email: fazelin931@mums.ac.ir

Background: Preconception care is essential in promoting pregnancy outcomes. Considering the low prevalence of preconception care and important role of health volunteers, current researchers conducted this study.

Objective: This study aimed to determine the effect of preconception care education based on the Health Belief Model on knowledge and attitude of women.

Materials and Methods: This randomized controlled trial with pretest-posttest design carried on 22 health volunteers and 110 married women aged 15-49 years old with an intention to become pregnant. Health volunteers in the intervention group were educated by the researcher according to the educational content of the preconception cares based on the Health Belief Model constructs and they educated women in the intervention group in three sessions of 90 minutes. Health volunteers in the control group were educated by the instructor of health volunteers and they educated women in control group with conventional manner. Data analyzed and significant level of $p<0.05$ was considered.

Results: The intervention and control groups were homogeneous according to age, pregnancy history and age of the youngest child. The results showed that the knowledge score with controlling the interventional variables including education, and baseline knowledge and perceived severity in the intervention group was 5.22 higher than the control group, and this difference was statistically significant ($t=8.14$, $p<0.01$).Attitude score with controlling of interventional variables including baseline perceived attitude and perceived severity in intervention group was 2.7 more than of control group ($t=2.99$, $p<0.01$). Comparison of the mean difference between the total score of the model after training compared to before, in the intervention group was 6.22 higher than the control group and this difference was statistically significant($t= 4.7$, $p<0.001$).

Conclusion: Education based on the Health Belief Model was more effective than conventional education in increasing knowledge and attitude. Therefore, educational interventions using Health Belief Model in promoting knowledge and attitude of women about preconception care is recommended.

Key words: *Preconception care, Knowledge, Attitude, Health belief model, Health volunteers.*

P-132

The relationship of the psychological coping and adjustment strategies of infertile women with the success of assisted reproductive technology

Golmakani N[1], Ebrahimzadeh S[1], Esmaily H[2], Kabirian M[3].

1. *Department of Midwifery, Nursing & Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Biostatistics, Social Determinants of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*
3. *Department of Midwifery, Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.*

Email: kabirianm1@gmail.com

Background: The success of assisted reproductive techniques plays a very important role in the quality of life of infertile couples and decreases the negative behavior states of infertility.

Objective: This study aimed at determining the relationship of different psychological coping and adjustment strategies with the success of assisted reproductive technology.

Materials and Methods: This correlational study was conducted on 204 women visiting Milad Infertility Center in Mashhad during 2015-2016. The research instruments included Fertility Adjustment Scale and Infertility Coping Strategies Scale. The positive results of 2 pregnancy tests within 48 hours was considered as the success of assisted reproductive technology.

Results: The mean and standard division score of adjustment in the group achieved treatment success (34.3±8.2) exceeded the group failed (33.6±8.8), the difference was not statistically significant ($p=0.381$). Also there was no significant difference between groups in the median and interquartile range of total coping strategies 81 (13) vs. 79.5 (12.25), ($p=0.369$), but the successful group had a higher coping score in the fate domain than the unsuccessful group ($p=0.037$). Based on the logistic regression model for one increased transferred embryo, the chance of getting pregnant is 1.3 times and for each unit increase in FSH level, the chance of ART success decreases 18%.

Conclusion: The results of this study can ensure that the infertile women are not concerned about choosing their coping and adjustment strategies, because these would

not affect their treatment success rates. It can also reduce the level of stress and anxiety of infertile women and improve their quality of life.

Key words: *Infertility, Psychological adjustment, Coping strategies, Assisted reproductive technology (ART).*

P-133

Evaluating the effectiveness of group and telephone counseling on sexual function after childbirth

Bokaie M[1], Hajimaghsoudi S[2], Dehghani A[3], Hosseini F[3].

1.*Department of Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: samira_hajimaghsoudy@yahoo.com

Background: Pregnancy and childbirth is a definite period of a woman's life that is associated with hormonal and physical changes in a person. These changes can have significant effects on the performance and sexual satisfaction of the individual. So far, sexual health counseling by telephone has not been conducted in Iran.

Objective: This study was aimed at the effect of group and telephone counseling on sexual function of lactating women.

Materials and Methods: This study was performed in 2017 on 54 women aged 18-45 years old in Yazd health care centers between 3 and 6 months after delivery. Entry criteria includes married Iranian Muslim women who are at least 18 years of age whose first marriage was the sole wife of a person and lived side by side, primiparous, have a lactation, their child is healthy, monogamous, terminated, due to normal delivery, access Was on the phone. The research units who entered the study were randomly assigned to two groups of intervention group and telephone counseling and control groups before the completion of the questionnaires. Demographic characteristics, and Sexual Function Questionnaire (FSFI) were used. The intervention group received 5 counseling sessions within 5 weeks with a one-week interval. Immediately after the end of the intervention and one month later, the sexual function questionnaire was again distributed and the results were evaluated. Data analysis was performed using SPSS software (version 16).

Results: The results showed no significant difference between the variables in two groups. The comparison between the intervention group and the control group showed significant increases in sexual function score ($p<0.05$).

Conclusion: The results of the study showed that group counseling and telephone counseling were effective, but telephone counseling was more effective. Therefore, it is recommended that the midwife counselor in the field of sexual health be able to serve women during postpartum and breastfeeding.

Key words: *Sexual health, Sexual satisfaction, Sexual function, Postpartum period.*

P-134

Comparing the effects of nursing versus peer-based education methods on the pre-operative anxiety in infertile women: A randomized clinical trial

Kalantari A[1], Farnia F[2], Aflatoonian A[1].

1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Nursing, Nursing-Midwifery School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: kalantariathara@gmail.com

Background: Pre-operative anxiety is a common event in patients expecting surgery. Training can play an important role in reducing the negative effects of anxiety on the response to treatment. Therefore, identifying the appropriate method is important.

Objective: The aim of this study was comparing the effects of nursing versus peer-based education on the pre-operative anxiety in infertile women.

Materials and Methods: In this clinical trial, 198 eligible infertile women were randomized into three groups: the nurse-educated, peer-educated, and the controls. The Spielberger State-Trait Anxiety Inventory (STAI) was filled out by all participants for measuring the patient anxiety at the time of hospital admission and the prior to surgery. Participants in the nurse-educated and peer-educated groups received a group education program by a nurse or peer, respectively, after the initial completion of the STAI. The control group was only educated at the time of their discharge which is part of the section's routines.

Results: The mean score anxiety was 44.47, 46.92, and 42.60 at the time of hospital admission and 39.38, 41.06, and 43.42, prior to surgery in nurse-educated, peer-educated, and the control groups, respectively. There was a significant difference in the mean score anxiety in each group before and after the intervention ($p<0.0001$). However, the difference among the groups was not significant.

Conclusion: Our findings demonstrate that the nursing and peer education programs reduce the pre-operative anxiety. Hence, making benefit of the peer's potential regarding the compensation for staff shortage for pre-operative education as well as investigating the effect of individual education is suggested for the further studies.

Key words: *Education, Infertility, Anxiety.*

P-135

Legal issues related to embryo donation and In vitro fertilization

Seyfipoor E[1], Seyfipoor M[2], Seyfipoor M[3].

1.*Department of Nursing and Midwifery, Shahid Beheshti Hospital, Isfahan, Iran.*
2.*Islamic Azad University, Tehran Branch, Tehran, Iran.*
3.*Islamic Azad University, Brogen Branch, Brogen, Iran.*
Email: elhmseyfioor@gmail.com

Donating embryo to couples with infertility has been allowed in the Iran Law pursuant to the embryo donation method Act ratified on 19 May 2013 and Administrative Regulation ratified on 09 Mar 2005. Embryo donation Act in the Iran Legal system is considered as new though and revolution and this new act is in the early administrative stage. So its benefits and disadvantages will be cleared in the future. The present article has studied in the field of legal issues related to embryo donation and In vitro fertilization and also problems of Iran Act in this regard. According to the Article 1 of embryo donation method to couples with infertility Act, specific centers are only allowable to transfer the embryo produced by In vitro fertilization in legal and juridical couples. There are two theories about In vitro fertilization with foreigner's sperm and wife egg that are pointed in the text. In compliance with article 3 of Administrative Regulation of embryo donation to couples with infertility method Act, embryo donation must be performed with written agreement and satisfaction of husbands who donate. Some jurists have stated that contracts that cause exchange of gamete and embryo are canceled and invalid because productions of human body principally do not must be treated property and good and subject of transaction. What is important in the law is rationality and Legitimating of the motivation not its origin. In this case since purpose and motivation is treating infertility through legally so it can be concluded that gamete is exchangeable but about embryo the separation must be allowed: 1- embryo composed of sperm and egg of couple that is maintained in the experimental environment. 2- embryo that is put into the uterus. in regard to the nature of the embryo donation contract division between its different stages must be allowed: the first stage that people deliver their gamete to the remedial centers can be based on the forgiveness, achate contract , peace etc and the second stage that the gamete is inoculated in the laboratory but yet is not placed into the uterus is also same as the first stage and the third stage that the embryo is transferred to the uterus predicating some titles like forgiveness, achate contract, peace etc. disagree with the human dignity and must be known as a private contract in the process of infertile couples treatment. descent of the child resultsed from the embryo transfer, in the Iran law only duties and tasks of couples who receive embryo have been stated in terms of maintenance, training, alimony, respect and they are known as duties of parents. Article 3 of embryo donation to parents method Act, has allowed the receiver couples to custody and training the child. There are some factors In the Islamic law and Fiqh that cause the reverence of matrimony between woman and man including descent, foster, blood familial, etc. that factor of descent and foster find subjectivism in the issue of embryo donation. According to regulation of the civil Cod, applicant husband is responsible for charity in the first stage and then wife is responsible if the husband is not applicant or lack of financial ability. The heredity relationship is just present between the child and sperm and egg holders and the Iran law has not stated about other descent effects. Of cases that must be noted in the Embryo donation method Act are further explanation of children rights resulted from performing this act including descent, alimony etc. method of meeting their costs in case of the couples is dead or occurrence of the divorce, considering some points including method of health Authentication, nationality, parity of donators, registration of embryo holders identity, method of classification and maintaining information of donators and receivers, registering natural identity of the child, the child's right to know about his/her real identity, borrowing uterus and overally reproduction or fertilization for other, transferring embryo after the husband death and administrative obligations and guarantee for regulations of donation and transferring embryo also in the law. We hope the obtained results by this research is effective in modification of regulations and writing essential regulations.

Key words: *Embryo donation, In vitro fertilization.*

P-136

Resilience as the predictor of quality of life in the infertile couple as the most neglected and silent minorities

Vatanparast M[1], Royani Z[2], Yaghmaie F[3].

1. *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Department of Paramedicine, Golestan University of Medical Sciences, Gorgan, Iran.*
3. *Department of Nursing, Zanjan Branch, Islamic Azad University, Zanjan, Iran.*

Email: mahboob_vatan@yahoo.com

Background: There is a new definition for Fertility Quality of Life (FertiQOL) that evaluates, in particular, the impact of infertility on various aspects of life. The inability to have children is experienced by couples as a heavy distress and often a heartbreaking situation. It is claimed that infertility plays a major role in the infertile population such as lower quality of life, mental health problems, emotional distress and marital and sexual dissatisfaction.

Objective: In addition, distress levels can influence the outcome of fertility treatment.

Materials and Methods: 202 infertile couples completed a questionnaire while waiting for their medical treatments. The questionnaire had three parts: Demographic characteristics, Quality of life of infertile couples (Ferti QOL), Connor-Davidson Resilience Scale (CD-RISC). The analysis was done via SPSS 20. Descriptive statistics were used for describing socio-demographic and clinical variables and independent sample t-test and χ^2 tests were used for comparing the means. Linear multiple regressions were conducted to examine whether resilience score had predictive value for men-women's QOL. Significant correlation coefficient indicate a significant relationship between the independent variables (resilience) and QOL scores for each domain. The $p<0.05$ was considered statistically significant.

Results: Overall mean score for QOL is higher in men compared to women (p>0.001), this score for men interpreted as positive (69.48% of total score) and neutral in women (58.87%). men presented a higher mean score in the six QOL domains scores, while women were superior only in physical domain and all differences were significant. The mean score of males' resilience is more than the females too (p=0.009). Resilience (p=0.04 and p=0.04) had significant prognostic coefficients for quality of life. In compare QOL score in terms of education, causes of infertility, and duration of infertility, the results was significantly different only in the education level.
Conclusion: The resilience for men and women can be regarded as a significant predictor for the most aspect of the QOL. Infertile couple with low resilience placed them as a risk group. Consultants must be considered in mind, the negative impact of the ART treatment on QOL of infertile men and women and provide a family-centered service in holistic approach.
Key words: *Quality of life, Infertility, Resilience.*

6- Stem Cell

P-137
Evaluation the effect of the conditioned medium from human testicular sperm extraction cell cultures on in vitro maturation in mice

Adib M[1], Seifati SM[1], Dehghani Ashkezari M[1], Khoradmehr A[2], Rezaee-Ranjbar-Sardari R[1], Mazaheri F[2], Akyash F[3], Aflatoonian B[2, 4].
1.*Medical Biotechnology Research Center, Islamic Azad University, Ashkezar Branch, Ashkezar, Iran.*
2.*Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: b.aflatoonian@ssu.ac.ir

Background: It was shown previously, that conditioned medium from mouse testicular cells induces female germ cell development in embryonic stem cells (ESCs) in several mammals including mice and Buffalo. Lacham-Kaplan and co-workers claimed the formation of artificial ovarian structures containing oocyte-like structures following culture with testicular conditioned medium. Similar data using gene expression profile assessment was shown in Buffalo ESCs. These findings indicate that factors secreted by testicular cells may support in vitro maturation (IVM) in mammals.
Objective: Here the impact of human testicular cell conditioned medium (TCCM) on mouse oocyte in vitro maturation was investigated.
Materials and Methods: 240 germinal vesicle (GV) oocytes were obtained from NMRI mice, aged 4-6 weeks that injected 5IU PMSG 48 hours before. GV oocytes without cumulus cells were subjected to in vitro maturation in TCCM (test group; 120 GVs) and DMEM+10%FBS (control group; 120 GVs). GV maturation rate was checked by microscopic observation at 8, 16 and 24 hr following IVM in both groups. The MII (metaphase II) oocytes were subjected for IVF. The fertilization rate was evaluated after 24, 48 and 72 hr.
Results: There was a significant difference between the maturation rates in TCCM (31.67%) and control (0%; p<0.05). IVF success rate for MII oocytes obtained in TCCM group was 28.94%.
Conclusion: Our data confirms previous findings which shown there are supportive factors in TCCM for female germ cell development. For further investigations we aim to evaluate the effect of TCCM on human IVM and assess the metabolomics profile of TCCM in the future.
Key words: *Conditioned medium, Germinal vesicle, Testicular sperm extraction, In vitro fertilization, In vitro maturation.*

P-138
Stem cells: A novel hope in cell-based therapy of infertility

Amini A[1], Tahmasbpour E[2], Nejad Moghaddam A[3].
1.*Department of Genetics, College of Science, Shahid Chamran University, Ahvaz, Iran.*
2.*Laboratory of Regenerative Medicine and Biomedical Innovations, Pasteur Institute of Iran, Tehran, Iran.*
3.*Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
Email: mab.biology@gmail.com

Background: Infertility is a prevalent condition that has insidious impacts on couples, their life, and society, which extend far beyond the inability to have a biological child.
Objective: In recent years, there have been several advances in the diagnosis and treatment of male infertility. However, current assisted reproductive techniques (ART) have failed to help infertile patients with non-obstructive azoospermia, unless donor sperm is used. During past few years a considerable progress in the derivation of male germ cells from pluripotent stem cells has been made.
Materials and Methods: PubMed was searched for all experimental and clinical studies using the keywords "stem cells", "male infertility", "spermatogenesis", "embryo development" and "reproductive function".
Results: Because of self-renewing and high differentiating potential of stem cells, they can be considered as new therapeutic agents for the treatment of infertility for individuals who don't produce gametes to have a biological child. Stem cells can be stimulated in vitro to develop various numbers of specialized cells, including male and female gametes suggesting their potential use in reproductive medicine.
Conclusion: This area of scientific endeavor holds great hope for infertile men wanting to father their own children. There seems to be every possibility that human male infertility can be reversed with stem cell research.

Although the use of embryonic stem cells (ESCs) is connected with many ethical concerns, there are no ethical issues regarding the use of induced pluripotent stem cells (iPSCs). Moreover, ESCs are genetically unrelated to the patients, while it may be possible to get offspring with their own genetics by using iPSCs in derivation of functional male gametes. However, the molecular mechanisms underlying human male germ cell development are still poorly understood.

Key words: *Stem cell, Infertility, Treatments.*

P-139

Fabrication and characterization of decellularized placenta-derived 3D bio-scaffolds for spermatogonial stem cells culture

Asgari F[1], Koruji M[1], Asgari H[1], Najafi M[2], Shabani R[1], Nikniaz H[1], Gholipour Malekabadi M[3], Bashiri Z[1].

1.*Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2.*Department of Biochemistry, Medical School, Iran University of Medical Sciences, Tehran, Iran.*
3.*Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Email: koruji1@gmail.com

Background: Male infertility affects 7 % of the male population and 10 % of infertile men are azoospermic. Extracellular matrix (ECM) of tissues contains various ranges of growth factors, proteins, proteoglycans, hyaluronic acid and others. Decellularization of tissue and production of tissue engineering scaffolds from ECM components is one of the most reliable strategies in fabrication of scaffolds. Although various protocols have been developed to remove the cells from tissues with minimal degradation of ECM components, an optimal protocol is still needed to satisfy scientists.

Objective: In this study, we decellularized human placenta and optimized a reliable protocol for spermatogonial stem cells (SSCs) in vitro culture.

Materials and Methods: Human placenta was obtained from mothers undergoing cesarean after obtaining informed consent. The tissues were treated with sodium dodecyl sulphate (SDS) for 30 min. The decellularized tissues were casted, freeze and freeze dried to fabricate a porous scaffold. The removal of the cells from tissues was determined by H&E and 4',6-diamidino-2-phenylindole (DAPI) staining, and DNA content assay. Alcian blue, Masson's trichrome and Orcein staining was used to confirm that ECM remained intact after decellularization process. Morphology of porous scaffold was viewed under scanning electron microscopy (SEM). SSCs viability for scaffold were determined by MTT assay.

Results: Histological analysis showed that the 0.5% SDS for 30 min was completely decellularized. Decellularization was further confirmed by DAPI staining and DNA content assay. Collaegen, elastin and glycosaminoglycans remained intact after decellularization process. MTT test showed no SSCs viability changes for the decellularized tissues.

Conclusion: Our study proved a reliable and effective protocol for decellularization of placenta with minimal negative effects on ECM components. The decellularized placenta is suggested as a promising bio-scaffold tissue engineering application that could improve attachment of SSCs.

Key words: *Decellularized Scaffold, Placenta, Extracellular matrix.*

P-140

Ovine endometrial cell culture on nanofiber scaffold: an approach for uterine tissue engineering

Dehghan M [1, 2], Nikukar H[2, 3], Khajeh-Mehrizi M[1].

1.*Textile Department, Faculty of Engineering, Textile Collage, Yazd University, Yazd, Iran.*
2.*Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: mahdiyeh.dehghan60@gmail.com

Background: In this study, a novel polycaprolactone/ gelatin/ polydimethylsiloxane (PCL/G/PDMS) scaffold with improved mechanical properties and controllable porous structure was prepared through electro spinning method. Good functional uterine endometrial cells are vital for fertilized egg implantation, repair, regeneration and normal activity of the uterus. This study introduced endometrial cell primary culture in vitro and cell culture on PCL/G/PDMS scaffold as the initial step for uterine tissue engineering.

Objective: Produced scaffold can be used in any case to repair the endometrial damage, in order to provide a good environment for implantation of the embryo and to reduce the infertility in the future. Using tissue engineering to create and reconstruct the uterine endometrium is one of the best reliable option for this purpose.

Materials and Methods: PCL, G and PDMS were purchased from Sigma-Aldrich® and trifluoroacetic acid (C2HF3O2) from Merck® companies. A PCL/G/PDMS copolymer was synthesized with volume ratios of difference (To fabricate scaffolds with optimum the diameter of nanofibers, porosity, hydrophilicity, biodegradability, biocompatibility, stress and strain of scaffolds, the effect of PCL/G/PDMS mixture ratio influencing the scaffolds characteristics were investigated via RSM). Fibers were electrospun onto an aluminum foil. The designed scaffolds were evaluated by a scanning electron microscope (SEM). Endometrial tissue samples from the uterus were collected in a sterile condition from five sheep after slaughtering in a slaughterhouse. Endometrial cells were isolated from endometrial samples. Isolated cells were cultured in cell culture medium. After sterilization under UV light for 20 min, to enumerate the cells, the population doubling time (PDT) was determined and 10^4 cells were cultured into 24-well

culture plates containing scaffolds. The cells were incubated in a humidified standard incubator at 37°C with 5% CO_2. Cells were harvested at days 3, 7, and 10 for growth and evaluated using H&E staining and MTT test. By triplicate assay the activity and survival of the cells on nanofibers were calculated as a percentage of samples to controls. In this study, the random scaffold was designed for the uterine endometrium layer and parallel scaffold for the uterine myometrium layer. Also, the diameter of nanofibers, porosity, hydrophilicity, biodegradability, biocompatibility, stress and strain of scaffolds have been studied.

Results: The SEM results showed that diameter of the fiber is at the nanoscale. The PCL/G/PDMS scaffolds significantly promoted endometrial cell attachment and proliferation comparing to PCL scaffold alone. Endometrial cell viability Was acceptable by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay. The H & E results also showed that the cells developed and replicated well on the scaffold. endometrial cells have grown on the parallel scaffold in line with nano fibers and on the randomistic scaffold have grown in all directions. This indicates that the cells are compatible with the Nano-fiber scaffolds produced. All quantitative and qualitative parameters indicate that the production scaffold is suitable for tissue engineering of the uterus.

Conclusion: These findings could be used in tissue engineering as the first step for uterine tissue engineering. Also, PCL/G/PDMS scaffold can be candidates for other regenerative cell therapy and tissue engineering.

Key words: *Tissue engineering, Nanofiber, Elastin, Ovine endometrial Cells.*

P-141

Improvement of primordial germ cells derivation from embryonic stem cells and conduce them to enter meiosis: An engineering approach

Esfandiari F.
Stem Cells and Developmental Biology Research Center, Royan Institute for Reproductive Biomedicine ACECR, Tehran, Iran.
Email: esfandiari65f@gmail.com

Background: Disruptions in germ cells (GCs) development or function cause infertility which is a major medical problem. However, current knowledge about the various mechanisms that underlie GC biology is still in its infancy. This lack of knowledge is primarily attributed to the low numbers of GCs in vivo which is an obstacle in GC research. Embryonic stem cells (ESCs) hold great promise for the production of an unlimited source of GCs. However, inability of ESC-derived GCs to go through meiosis in vitro remains a challenge. We hypothesized that insufficient diffusion of soluble factors from culture media toward inside the aggregates cannot provide effective signals for efficient GC differentiation from ESCs.

Objective: To address this issue, we have developed bone morphogenetic protein 4 (BMP4)-releasing microparticles (MPs) from alginate sulfate and incorporated them within the aggregates to provide a controlled inside-out delivery of BMP4.

Materials and Methods: We synthesized alginate sulfate and characterized it by FTIR. MPs were produced from alginate sulfate and BMP4 was loaded in the MPs. The incorporation of MPs within aggregates was assessed. The BMP4-laden MPs were applied during GC differentiation from ESCs. Then, we used immunofleurescence immunohistochemistry and q-RT PCR in order to analysis the BMP4-laden MPs function during PGCLC differentiation from ESCs.

Results: Our results showed that affinity-based delivery of BMP4 in a sustained and localized manner increased GC differentiation from ESCs at least two-fold compared to the conventional soluble delivery method. Interestingly, following meiosis induction, we have observed that Dazl, an intrinsic factor that enables GCs to enter meiosis, and two essential meiosis genes (Stra8 and Smc1b)were upregulated significantly in MP-induced aggregates compared to aggregates which formed by the conventional method. MP-induced aggregates formed tube-like structures similar to seminiferous tubules of the testes.

Conclusion: Together, these data showed that controlled delivery of BMP4 during ESC differentiation into GC established meiosis-competent GCs, which could serve as an attractive GC source for reproductive medicine.

Key words: *Microparticles, Germ cells, Meiosis, Infertility.*

P-142

Influences of mesenchymal stem cells differentiate to bone, cartilage and fat cells after knocking down of Toll-like receptors

Khodabandehloo F[1], Nassiri-Asl M[2], Rajaei F[3], Zandieh Z[4], Baghaban Eslaminejad M[5], Aflatoonian R[6].
1. *Department of Molecular Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.*
2. *Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.*
3. *Fertility and Infertility Research Centre, Qazvin University of Medical Sciences, Qazvin, Iran.*
4. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
5. *Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biologyand Technology, ACECR, Tehran, Iran.*
6. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, Tehran, Iran.*

Email: R.Aflatoonian@gmail.com

Background: Mesenchymal stem cells (MSCs) are multipotential stem cells that can differentiate into osteoblasts, chondrocytes and adipocytes. MSCs thought to be excellent candidate tools for the field of regenerative medicine, due to their differentiation potential. Therefore, it is important to reveal the molecular mechanisms that regulate the MSC function including survival, proliferation and differentiation. TLRs, which expressed in MSCs, are critical in regulating the fate decision of MSCs. In addition to

pathogen-associated molecular patterns, a variety of endogenous agonists recognized by TLRs.
Objective: In this study, we investigated the role of TLRs activation (both endogenous and exogenous) on differentiation potential of mesenchymal stem cells into three lineages. We also aimed to investigate the effect of TLRs knockdown on the differentiation of human mesenchymal stem cells (MSCs) from bone marrow.
Materials and Methods: MSCs were stimulated with endogenous and exogenous ligands. TLR3 and TLR4 expression was knocked down by psiRNA. The differentiation potential of MSCs was evaluated in adipogenic, chondrogenic and osteogenic differentiation media by gene expression related to the differentiation at day 7, 14, and 21. TLRs mRNA expression assessed by Real Time PCR in the treated and control groups.
Results: The regulatory role of TLRs was identified on mesenchymal stem cell differentiation and was found that activated TLRs by their ligands promote the osteogenic and adipogenic differentiation of MSCs in vitro.
Conclusion: These results suggested that TLRs regulates bone marrow MSC adipogenic and osteogenic differentiation. These data also provide new insight in developing new therapy in bone regeneration using MSCs by toll-like receptors ligands.
Key words: *TLRs, Knockdown, Human mesenchymal stem cells, Differentiation, Gene expression, Ligands.*

P-143
Human amniotic membrane successfully decellularization using of SDS

Haghshenas M, Tavana S, Montazeri L, Fathi R.
Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: rfathi79@yahoo.com

Background: Owing to its unique biological composition and regenerative properties, the human amniotic membrane (hAM) has been used effectively in the clinical grafting applications.
Objective: In the present study, hAM was decellularized using sodium dodecyl sulfate (SDS) treatment to provide a three-dimensional (3D) bioscaffold.
Materials and Methods: The qualitative histological evaluations including hematoxylin and eosin (H&E), Masson's Trichrome (MT, for collagen assay), 4,6-diamidino-2- phenylindole (DAPI), and Alcian blue (AB) staining (for glycosaminoglycan (GAG) assessment), as well as the quantitative assessments including nuclear DNA contents and GAGs assays, were performed on 3D bioscaffolds to confirm the absence of cell nuclei in the decellularized hAM (dhAM) treated with SDS (1%) in comparison to the control group (intact human amniotic membrane (IAM)).
Results: H&E and DAPI staining proved that hAM cells were removed from dhAM. MT and AB staining revealed that the GAGs and collagen fibers respectively were preserved in dhAM when compared to the IAM group. Quantitative assays showed that the DNA and GAGs content of dhAM was significantly reduced as compared to the IAM group (114 vs. 6207 ng/mg for DNA and 166 vs. 270 ng/mg for GAGs content; $p<0.01$).
Conclusion: Decellularization of hAM by means of the SDS (1%) is an efficient method to provide a three-dimensional bioscaffold for using in tissue engineering.
Key words: *Human amniotic membrane, Decellularization, SDS.*

P-144
Human bone marrow mesenchymal stem cells in vaccine production: The role of TLR4-LPS axis

Hashemzadeh M[1], Aflatoonian R[2].
1.*Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
2.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: r.alatoonian@gmail.com

Background: Lipopolysaccharides (LPS) from gram negative bacteria stimulate toll-like receptor 4 (TLR4) expressions in immune cells. Recent reports state that bone marrow-derived cells such as mesenchymal stem cells (MSCs) also express TLR proteins. Numerous researches have studied the effect of a number of LPSs on TLR4 expression, but no data exists on the effect of LPSs from different strains of one bacterial genus on TLR4 expression.
Objective: Numerous researches have studied the effect of a number of LPSs on TLR4 expression, but no data exists on the effect of LPSs from different strains of one bacterial genus on TLR4 expression.
Materials and Methods: In this study, we investigate the effects of various concentrations of LPS from different Shigella strains on TLR4 expression in human bone marrow (hBM)-MSCs. At the mRNA level, we have found that untreated hBM-MSCs (control) did not express TLR4 compared to the experimental groups. Cells treated with LPS from Shigella flexneri had the highest expression of TLR4, whereas cells treated with LPS from Shigella sonnei had the lowest expression.
Results: We observed that LPSs had a dose-dependent effect on TLR4 expression in all of the treatment groups. ELISA findings for interleukin-6 secretion have confirmed mRNA expression results for all treatment groups.
Conclusion: According to the data, it can be obviously inferred that the LPS from S. flexneri can be considered as an optimum LPS to stimulate the immune system for vaccine production against shigellosis. Moreover, the axis of TLR4-LPS can be studied in the modulation of homing behavior of hBM-MSCs in the way of stem cell therapy for cellular degenerative diseases.
Key words: *Human bone marrow mesenchymal stem cells, LPS, Shigella, TLR4.*

P-145
Amniotic fluid derived mesenchymal stem cells and their potential applications in regenerative medicine

Hoseini SM[1], Kalantar SM[2], Aflatoonian B[3], Bahrami AR[1], Moghaddam-Matin M[1].
1.*Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: matin@um.ac.ir

Amniotic fluid contains a mixture of different cell types sloughed from the fetal skin, respiratory, alimentary and urogenital tracts, as well as the amnion membrane. As amniotic fluid develops prior to the process of gastrulation, many cells found in its heterogeneous population do not undergo lineage specialization. Therefore, amniotic fluid-derived mesenchymal stem cells (AF-MSCs) may correspond to a new class of stem cells with properties of intermediate plasticity between pluripotent and adult stem cell types. They have some unique features which make them fascinating for researchers, such as differentiation into cells derived from three germ layers, high clonal capacity, ability to form embryoid bodies, expression of pluripotent markers, high self-renewal capacity (over 250 population doublings) with normal karyotype at late passages, long telomere length due to continued telomerase activity, specially non-tumorigenicity, low immunogenicity, anti-inflammatory and immunomodulatory properties for their applications in regenerative medicine.
In comparison with mesenchymal stem cells (MSCs) from other sources, such as bone marrow, AF-MSCs show higher proliferation and differentiation capacity. Since 2001, when for the first time AF-MSCs used in tissue engineering (cultured on polyglycolic acid poly-4-hydroxyapatite scaffolds), many researchers have published a wide range of reports about using AF-MSCs in regenerative medicine and tissue engineering. For example, AF-MSCs have been used for regeneration of neural tissue (making construct with AF-MSCs and fibrin glue for regeneration of the sciatic nerve) and lung regeneration (integration of AF-MSCs among lung mucosal cells and expression of alveolar and bronchiolar markers by transplanting them into an injured murine lung). Moreover, it has also been shown that AF-MSCs have a great potential in cardiac regeneration, such as the optimistic results obtained through injection of these cells directly into the peri-infarct areas in rat myocardial infarction model. In summary, it is predicted that these cells will be one of the most interesting stem cell sources for regenerative medicine applications in the future.
Key words: *Amniotic fluid, AF-MSCs, Regenerative Medicine, Stem cell.*

P-146
The effect of 3D Scaffold and microfluidic culture system on spermatogonial stem cells proliferations

Moeinzadeh A[1], Ashtari Kh[2, 3], Koruji M[1, 2], Shabani R[1, 2].
1.*Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
2.*Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.*
3.*Department of Medical Nanotechnology, Faculty of Advanced Technology in Medicin, Iran University of Medical Sciences, Tehran, Iran.*
Email: shabani.r@iums.ac.ir

Background: Some male survivors of childhood cancer are in distress from azoospermia. Spermatogonial stem cells (SSC) isolation and purification are really important. Testicular organoids and 3D scaffolds are possible applications in treatment of male infertility. 3D nanofibers scaffolds play important role in cell culture, due to these scaffolds provide a microenvironment similar to extracellular matrix for proliferation and self-renewal of cells. A micro fabricated chip made of polydimethylsiloxane (PDMS) and having a set of micro-channels etched or molded into it, can serve as a culture vessel for cells and can easily accommodate medium few or Laboratory of Proteomics.
Objective: The present study aimed to evaluate efficiency of 3D microenvironment containing the chitosan-alginate -Graphene oxide nanocomposites and microfluidic culture system for improve cell culture and proliferation of spermatogonial cells.
Materials and Methods: Spermatogonial cells were cultured and divided into 5 culture groups: 1/ Control (culture in basic media), 2/ SSC culture in CA/GO scaffold, 3/ SSC culture on CA/GO scaffold in microfluidic system, 4/ cells culture on GO nanocomposites and 5/ the cells culture on alginate-chitosan. The identity of the cultured cells was confirmed by flow cytometry (ckit and GFRa1). The Scaffolds were investigated by SEM to observe surface topography and the morphology. Cytotoxicity of scaffold was assayed at 24h, 72h and one week after seeding using MTT assay. The stem cells related markers for SSCs (Id4 ,GFRa1 and PLZF) were detected on all experimental groups by qRT-PCR.
Results: These results showed that SSCs can easily attach and proliferate on. The CA/GO scaffolds were biocompatible, as evidenced by the MTT assay. Spermatogonial stem cells that were seeded onto the surface of the scaffold exhibited good proliferation. The quantity of proliferations marker significantly increased compared with control group. The qRT-PCR results confirmed that GO-based/Alginate-chitosan scaffold may provide an ideal environment for SSC proliferations.
Conclusion: We conclude that SSCs culture in the group CA/GO/ MF have potential use for SSCs proliferation in vitro. This three-dimensional scaffold is applicable for culturing and encapsulation of spermatogonial stem cells.
Key words: *Spermatogonial stem cells, Graphene oxide nanocomposites, CA/GO scaffold, Microfluidic culture system.*

P-147
An investigation of the effect of the human cumulus cell conditioned medium on mouse oocytes in vitro maturation

Rezaee-Ranjbar-Sardari R[1, 2, 3], Adib M[1, 2, 3], Khoradmehr A[3], Mazaheri F[3], Tahajjodi SS[2, 3, 4], Aflatoonian B[2, 4,5, 6].

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

Email: b.aflatoonian@ssu.ac.ir

Background: Since defect of oogenesis is the cause of infertility in some infertile couples, *in vitro* maturation (IVM) of germinal vesicle (GV) oocytes is an important option in infertility treatments. Cumulus cells are essential for nutrition of oocytes during oogenesis. They provide a suitable environment contains growth factors required for oogenesis. It was shown that cumulus cell conditioned medium (CCCM) supports female germ cell development from Buffalo embryonic stem cells (ESCs).

Objective: In this study the impact of CCCM on murine IVM was investigated.

Materials and methods: NMRI female mice, aged 4-6 weeks, were injected with 5 IU of Pregnant Mare's Serum Gonadotropin (PMSG) intrapritoneally (IP). 240 germinal vesicle (GV) oocytes were obtained. GV oocytes without cumulus cells were cultured in CCCM (test group; 120 GVs) and DMEM+10%FBS (control group; 120 GVs). GV maturation rate was checked by microscopic observation at 8, 16 and 24 hours following IVM in both groups.

Results: Significant difference was observed between CCCM (19.16%) and control (0%; $p<0.05$) following IVM.

Conclusion: Human CCCM supports IVM in mice oocytes. For further investigations we aim to evaluate the effect of TCCM on human IVM and assess the metabolomics profile of TCCM in our future studies.

Key words: *Conditioned Medium, Cumulus Cells, Germinal Vesicle, In Vitro Maturation.*

P-148

An investigation of the effect of the human embryonic stem cells conditioned medium on mouse oocytes in vitro maturation

Rezaee-Ranjbar-Sardari R[1], Dehghani Ashkezari M[1], Seifati SM[1], Khoradmehr A[2], Adib M[1], Mazaheri F[2], Golzadeh J[2], Aflatoonian B[2, 3].

1.*Medical Biotechnology Research Center, Islamic Azad University, Ashkezar Branch, Ashkezar, Iran.*
2.*Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: b.aflatoonian@ssu.ac.ir

Background: Secreted factors inside the medium by the cells provide an appropriate environment in the conditioned medium to support the growth of other cells. It has been reported that conditioned medium from mouse embryonic stem cells (mESCs) supports in vitro maturation (IVM) of mouse oocytes.

Objective: In this study the impact of human ESCs (hESCs) conditioned medium (ESCM) was investigated on IVM of mouse oocytes.

Materials and Methods: NMRI female mice, aged 4-6 weeks, were injected 5 IU of Pregnant Mare's Serum Gonadotropin (PMSG) intrapritoneally (IP). 240 germinal vesicle (GV) oocytes without cumulus cells were cultured in ESCM (test group; 120 GVs) and HES medium (control group; 120 GVs). Following microscopic study to observe metaphase II (MII) development from GVs to evaluate the oocyte IVM rate for, at 8, 16 and 24 hours, to assess their fertilization capacity, the matured oocytes were undergone in vitro fertilization (IVF).

Results: Our data indicated a significant difference between the IVM rates in ESCM and HES ($p<0.05$), whereas IVF rate was not significant between two groups ($p>0.05$).

Conclusion: There are some factors secreted by hESCs in the culture medium which supports IVM in mouse. For the future study, we aim to evaluate the effect of ESCM on human IVM. Metabolomics study is in progress for understanding the details of these results.

Key words: *Conditioned Medium, Human embryonic stem cell, In vitro maturation.*

P-149

Chondrogenesis of human embryonic stem cells using a sequential 3D and 2D culture

Shahriary S[1, 2], Tahajjodi SS[3], Akyash F[3], Golzadeh J[1], Aflatoonian B[1, 2].

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

Email: b.aflatoonian@ssu.ac.ir

Background: Osteoarthritis (OA) is one of the most prevalent joint diseases around the world. Cell-based-therapies considered as useful approach for OA treatment. Autologous chondrocyte implantation (ACI) despite non-immunogenicity, can results in disrupting chondrocyte morphology and fibrocartilage formation. Hence, Due to their innate pluripotency, unlimited self-renewal and human source, human embryonic stem cells (hESCs) can be used as an appropriate cell source for OA cell therapy. According to the different reports, culture

methods using embryoid body (EB) formation (3D) and monolayer (2D) can be effective for hESCs chondrogenic differentiation. Chondrogenesis from hESCs can provide promising in vitro model for developmental biology studies, drug discoveries and ultimately regenerative medicine applications for human articular cartilage.
Objective: Here, chondorgenesis of hESCs was induced using a sequential 3D-2D culture system and assessed by alcian blue and trichrom staining.
Materials and Methods: Human ESCs (Yazd4; 46,XX) were induced for differentiation using EB formation in a non-adherent culture condition as 3D culture for 4 days. Then EBs were transferred and cultured further in adherent monolayer culture condition as 2D culture system till day 14 of differentiation. Differentiated EBs and cells were assessed for chondrogenesis induction using alcian blue and trichrom staining following 4 and 14 days of differentiation.
Results: Our data has indicated that sequential 3D-2D culture of hESCs induced homogenous population of chondrocytes as identified by alcian blue and trichrom staining following 4 and 14 days in culture.
Conclusion: Sequential 3D-2D culture of hESCs can induce spontaneously in vitro chondrogenesis. This method can be improved using defined culture condition and conditioned medium for more efficient outcome and further studies in cell-based therapeutic studies.
Key words: *Chondrogenesis, Embryoid body, Human embryonic stem cells, Monolayer culture.*

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P-150
Study of main genes involved in apoptotic pathway in testicular tissue of Wistar male rats induced by varicocele

Ahmadzadeh Golsefied A, Nasr-Esfahani MH, Tavalaee M.
Royan Institute for Biotechnology, Isfahan, Iran.
Email: mh.nasr-esfahani@royaninstitute.org

Background: According to the World Health Organization (WHO) Infertility is defined as the inability to achieve a pregnancy after one year of unprotected sexual intercourse. 10-15% of couples face infertility, which is related to male factors in almost 50% of cases. The most common causes of male infertility are varicocele. Varicocele refers to the abnormal inflation of spermatic veins in the scrotum sac. Studies have shown that varicocele is associated with an increase in apoptosis and therefore a reduction in the number of sperm.
Objective: The purpose of this investigation was to create a varicocele model in animals and to study the apoptosis gene expression and their effect on survival of sperm cells were evaluated.
Materials and Methods: In the present study, 30 adult male Wistar rats were divided into three groups, I: varicocele-induced II: sham and III: control. After two months of varicocele induction, rats were sacrificed and epididymis were dissected. Then, sperm Viability assessed by WHO protocol, and eosin-nigrosine test. In addition, Real Time PCR technique was used to determine the expression of the apoptosis gene of the RNA level. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Tukey). Collected data were presented as mean±SEM and $p<0.05$ was considered to be significant.
Results: The results of the current study showed that mean Sperm Viability were significantly lower in varicocele induction group compared to control and sham groups ($p<0.01$), while the level of apoptosis genes mRNA expression to be significantly higher in varicocele induction group compared to control and shame groups ($p<0.05$).
Conclusion: In the varicocele status due to the high testicular temperature, and consequently increase of oxidative stress level, apoptosis pathway genes such as P53, Bak, Bax, Caspase3 were activated and that lead to the cell death. Consequently, in individuals with varicoceles, the rate of apoptosis increases and sperm count have decrease. These conditions reduce the quality of semen and can lead to infertility in men.
Key words: *Infertility, Varicocele, Apoptosis, Reduces sperm count.*

P-151
Investigation the effects of Irisin on proliferation, invasion and apoptosis of ovarian cancer cells

Alizadeh Zarei M[1], Seyed Hosseini E[1], Haddad Kashani H[2], Amini Mahabadi J[1].
1.*Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
2.*Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
Email: marziyehalizadeh91@yahoo.com

Background: Irisin is one of the recently known novel myokines. The "myokines" are a family of hormones secreted by muscles. They participate in several physiological and pathological processes, for example, lipid metabolism and cancer progression. Currently, Irisin was proved to improve the quality of skeleton and its serum concentration in cancer patients was lower than healthy participants. Because of Irisin's potent metabolic effects on several tissue types, it is conceivable that Irisin may possess the ability to alter malignant features similar to other myokines. No previous studies have evaluated whether Irisin may regulate cell proliferation and malignant potential of ovarian cancer cell lines.
Objective: The aim of this study was to evaluate the effect of Irisin on ovarian cancer cells" behavior.
Materials and Methods: To determine whether Irisin has a protective effect against ovarian cancer, we cultured SKOV3 and OVCAR3 ovarian cancer cells and treated them with Irisin at different concentrations. We detected the proliferation by MTT and colony formation assays. Furthermore, we assessed the migration and invasion of the cells by Trans-well assay. The expression levels of

MMP2 and MMP9 as metastasis markers and a panel of genes related to Warburg effect were detected by real-time analysis. Meanwhile, flow cytometery was used to investigate the effect of irsin on apoptosis induction in ovarian cancer cells.

Results: We demonstrated that Irisin inhibited the proliferation and colony formation ability of ovarian cancer cells. Tran-swell assay revealed that Irisin also inhibited the migration and invasion of ovarian cancer cells. Additionally, we found that Irisin reduced apoptosis induction in ovarian cancer cell lines. Our data also suggested that the expression level of metastasis markers (MMP2 and MMP9) and some of the surveyed genes related to Warburg effect decreased after Irisin treatment.

Conclusion: Exercise has been well-documented to reduce cancer risk and improved prognosis of cancer patients. Our findings provide possible insights into potential mechanisms underlying these observations. Moreover, our data supports the hypothesis that Irisin may play a key role in future cancer therapeutics. However further in vitro and in vivo investigations are needed to support these data before using in clinics.

Key words: *Ovarian cancer, Irisin, Proliferation, Invasion, apoptosis.*

P-152

Comparison between gene expression SPAG11A in process of spermatogenesis in infertile men who suffering from varicocele (grade 1-2) pre- and post-treatment

Amiri S[1], Kalantar SM[2], Peymani M[1], Gholizadeh L[3], Rasti A[2, 4], Vahidi S[3], Mirjalili SAM[3].

1.*Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Email: ss.amiri1991@gmail.com

Background: Human sperm-associated antigen 11A (SPAG11A) gene is a member of the beta defensin family. This gene encodes SPAG11A protein, an epididymal protein, which interacts with the sperm surface, and is implicated in the sperm maturation and function. Varicocele, as one of the main causes of male infertility might cause a series of pathophysiological changes in the testis and epididymis, with an adverse effect on the environment for spermatogenesis and sperm maturation. It also leads to alterations in sperm characteristics and decrease in sperm parameters.

Objective: To evaluate conventional sperm parameters and expression of SPAG11A mRNA in the spermatozoa of men with varicocele grade I and II before and after treatment.

Materials and Methods: Twenty infertile men with varicocele grade I and II were enrolled in this study. Semen specimens were collected from subjects before and after varicocele treatment. Semen analysis was conducted in accordance with WHO guidelines. To assess the relative expression level of SPAG11A gene, total RNA was extracted for first-strand cDNA synthesis. Afterwards, the expression of SPAG11A mRNA was determined by quantitative real time PCR (qRT-PCR). The data were analyzed by Student's t test and Mann-Whitney U statistical method. $P<0.05$ was considered significant.

Results: No significant differences were found between the subjects regarding conventional sperm parameters pre- and post-treatment ($p>0.05$). However, the expression level of SPAG11A was significantly increased after treatment in comparison with its expression before treatment ($p<0.05$).

Conclusion: From the obtained data it could be concluded that the SPAG11A gene expression may be affected by varicocele. With respect to the remarkable role of SPAG11A protein in sperm epididymal maturation, it appears that varicocele treatment would be a good strategy to reduce adverse effect of this clinical problem on expression of SPAG11A.

Key words: *SPAG11A, Beta defensin, Varicocele, Epididymis, Quantitative real time PCR.*

P-153

Evaluation of expression level of *DGCR8* gene in non-obstructive azoospermic patients in compare with obstructive azoospermic patients

Babakhanzadeh E, Khodadadian A, Aghaei M, Talebi M, Mazaheri M.

Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: mazaheri54@yahoo.com

Background: DGCR8 (DiGeorge syndrome chromosomal region 8) is critical for the processing of pri-miRNA into pre-miRNA. Several experiments of knock out mice model revealed the vital role of DGCR8 in mice infertility. DGCR8 is also an essential element in the meiotic and haploid phases of spermatogenesis and transition from round spermatids to mature spermatozoa. The purpose of this study was to examine changes in DGCR8 gene expression in OA and NOA patient.

Objective: The purpose of this study was to examine changes in DGCR8 gene expression in OA and NOA patient.

Materials and Methods: This research investigated the expression pattern of DGCR8 gene in testicular biopsies of 60 infertile men who had undergone testicular sperm extraction (TESE) procedure. Samples were classified into five groups according to histopahtology: obstructive azoospermia (OA, n=15), early maturation arrest (eMA, n=11), late maturation arrest (lMA, n=10) and Sertoli cell only syndrome (SCOS, n=12) hypospermatogenesis (HP, 13). Relative expression of DGCR8 gene was measured by quantitative real-time polymerase chain reaction (qRT-PCR). One-way ANOVA analysis was used to determine inter-group differences in DGCR8 gene expression among the five histologic groups.

Results: Experiments including the detection of mRNA showed a severe decrease of DGCR8 in samples with eMA and SCOS compared with samples with OA ($p<0.05$, One-way ANOVA analysis).
Conclusion: The results of this study indicate that DGCR8 has a high expression in the germ cells, especially in the spermatocyte stage and possibly the early stages of miosis (mid/late pachytene) ($p<0.05$, One-way ANOVA analysis). Protein studies are necessary to determine the exact location of this gene expression and the separation of azoospermic subtypes, although the claim needs more samples to be confirmed.
Key words: *DGCR8, Spermatogenesis, Non-obstructive azoospermic.*

P-154
Curative treatment options for men with non-obstructive azoospermia

Babakhanzadeh E, Talebi M, Aghaei M, Khodadadian A, Mazaheri M.
Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: mazaheri54@yahoo.com

Background: Men with NOA have confined options for reproduction. Testicular sperm extraction followed by Intracytoplasmic Sperm Injection (ICSI) is feasible in these patients. But there are high costs and major problems for a female partner. If mature sperm cannot be recovered from the testes, Patients almost all lose hope in having a biological child. Therefore, the need for alternative therapies for the production of adult spermatozoa is necessary in patients with azoospermia. Many techniques are in the tentative stage to prepare this patient population alternatives for conceiving.
Objective: The purpose of this study is to introduce new techniques that can be used in the future for the treatment of azoospermia men.
Materials and Methods: This review considers three of the tentative techniques for returning fertility in men with NOA: spermatogonial stem cell (SSC) transplantation, in vitro spermatogenesis using adult or embryonic pluripotent stem cells, and gene therapy.
Results: SSC transplantation testicular tissue grafting retains the natural stem cell niche, Cryopreservation protocols, SSC injection is the other method (This process should theoretically permit for conception without the necessity of assisted reproductive techniques, that is a great benefit). Several problems have tortured the transformation of this notion into an actual clinical therapy for patients: recognizing the SSC population in the testis, culturing the SSCs, storing of the SSCs, and reintroducing the cells safely into a recipient. There are two stem cell sources (in addition to SSCs) that might be applied in producing germ cells: human embryonic stem cells and adult pluripotent stem cells. Until the other protocol is created, adult pluripotent stem cells are not an authority as a therapy for male infertility due to their tumorigenic risks. Gene therapy needs a perfect conception of the specific genetic defect underlying the pathologic method. Unfortunately, such wisdom is lacking in the most of the cases of NOA.
Conclusion: Ethical and technical restrictions create a significant obstacle to performing the suitable studies in humans and reliable reproducible protocols are essential.
Key words: *Non-obstructive azoospermia, Spermatogonial stem cell, Gene therapy.*

P-155
Overexpression of *c-Maf* gene in women with endometriosis

Bajool N[1], Shahhoseini M[2], Ramezanali F[3], Amirchaghmaghi E[4].
1.*Department of Cell and Molecular Biology, College of BioScience, Islamic Azad University North Tehran Branch, Tehran, Iran.*
2.*Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4.*Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
Email: amirchaghmaghi_e@yahoo.com

Background: Endometriosis is an inflammatory gynecologic disease in women of reproductive age. This disease is characterized as implantation and growth of endometrial tissues outside the uterine cavity. Women with endometriosis suffer from different signs and symptoms such as dysmenorrhea, chronic pelvic pain and infertility. The pathogenesis of endometriosis has not been completely understood. Several studies suggest that changes in immune responses in endometriotic milieus play an important role in pathogenesis of endometriosis. One of these alterations is change in different subsets of T helper (Th) cells. c-musculoaponeurotic fibrosarcoma (c-Maf) is a transcription factor that expressed in Th2, Th17 and T regulatory (Treg) which ultimately leads to the production of cytokines such as IL4, IL10 and IL21.
Objective: The aim of this study was to investigate the expression level of c-Maf in the endometrial tissues of women with endometriosis compared to control ones.
Materials and Methods: In this case control study, 13 women with endometriosis and 13 women without the endometriosis were enrolled in the study after diagnostic laparoscopy. Ectopic samples were collected through laparoscopic procedure and eutopic samples were collected using pipelle. The control endometrial samples were also obtained from normal endometrium by pipelle. The gene expression of c-Maf in normal, eutopic, and ectopic endometrial samples were evaluated quantitatively by real-time polymerase chain reaction (real time-PCR). Gene expression data were analyzed based on $2^{-\Delta\Delta ct}$ to estimate the relative fold change values. Data were analyzed by one-way ANOVA followed by Tukey's test. $P<0.05$ was considered as statistically significant.

Results: Based on the findings of this study, the mRNA expression level of c-Maf was significantly increased in ectopic tissues of patients with endometriosis compared to eutopic and control endometrial tissues ($p<0.05$). Although mRNA expression of c-Maf was higher in eutopic endometrial tissues of endometriosis women compared to control ones but this difference was not statistically significant ($p>0.05$).
Conclusion: These data collectively identified that c-Maf overexpression may be critically involved in development of endometriosis beyond its role in promoting T helper responses. For getting more information, larger sample size is needed. Also expression studies of other transcription factors involved in T helper cell differentiation are recommended.
Key words: *Endometriosis, T-helper cells, c-Maf, Ectopic, Eutopic.*

P-156
Reanalyzing discarded blastocyst embryos for autosomal aneuploidy after sex selection in cleavage stage

Ebrahimian N[1], Montazeri F[2], Sadeghi MR[1], Kalantar SM[2], Gilany K[3], Khalili MA[4].
1.*Reproductive Embryology and Andrology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research, Tehran, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
4.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences.*
Email: khalili59@hotmail.com

Background: Pre-implantation genetic screening (PGS) is used to identify common chromosomal abnormality. Aneuploidy is a key genetic factor that can influence human reproductive success in ART. Moreover, existence of high rate of mosaicism in cleavage stage has been reported. In spite of rescue from aneuploidy in blastocyst, evaluation of chromosomal abnormality is crucial.
Objective: Evaluation of autosomal aneuploidies in discarded embryos after sex selection which was received to the blastocyst stage.
Materials and Methods: Thirty discarded embryos which were biopsied in cleavage stage and reached to blastocyst stage reanalyzed by fish method for 8 chromosomes: 13, 15, 16, 18, 21, 22, xy. These discarded embryos were resulted from PGS program for sex selection.
Results: Although, it is expected that the rate of chromosomal abnormalities in the blastocyst stage would be reduced than cleavage stage. Our results were showed that a significant rate of autosomal aneuploidy in reanalyzed embryos which were reached to the blastocyst stage. Overall, the study of aneuploidies rate in the blastocyst stage indicated that 40% of the investigated blastocyst embryos had chromosomal abnormalities. Among the analyzed blastocyst for common 8 chromosomes, chromosome 13 have the highest rate of abnormality and aneuploidy in the other chromosomes ranged between 5-10%.
Conclusion: Our results suggest that, to be appraised critical autosomal chromosomes in combined with sex chromosome in a PGS program for sex selection. These chromosomes have a predominant role in aneuploidies detected in recurrent abortions, IVF failure and disruption occurs in the term.
Key words: *PGS, FISH, Trophoectoderm biopsy, ART.*

P-157
Quantitative evaluation of HILS1 expression and its potential interaction with regulating microRNAs in testicular tissue of infertile men

Goli N[1], Shahhoseini M[2].
1.*Department of Genetics, University of Sciences and Culture, Tehran, Iran.*
2.*Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: shahhoseini@royaninstitute.org

Background: Spermatogenesis is a highly dynamic process that is controlled by many factors including epigenetic regulators. Spermatid-specific linker histone H1-like protein (Hils1), a highly expressed protein in the nuclei of elongating and elongated spermatids, has been suggested to participate in spermatid nuclear condensation during spermiogenesis in mice. HILS1 gene does not encode any protein in humans, and might serve as a long non-coding RNA to exert its potentially important effects during human spermiogenesis, particularly because it is specifically expressed in testis and spermatozoa.
Objective: This study was aimed to evaluate the HILS1 transcript levels in testis and identify microRNAs potentially regulating this gene in testicular tissue of infertile men under testicular epididymal sperm extraction (TESE) operation.
Materials and Methods: Twenty-two samples of testicular biopsy from infertile men were obtained using TESE procedure, including five samples from patients with hypospermatogenesis (positive control), seven samples from patients with Sertoli cell only syndrome, and 10 samples from patients with complete maturation arrest at second spermatocyte level. The expression of HILS1 transcript was analyzed using qRT-PCR and GAPDH was used as the internal normalization control. Several microRNA software such as miRBase, RNAfold, miR-DB, and TargetScan were used to predict and investigate the potentiality of HILS1 to be either regulated by microRNAs or processed into microRNAs.
Results: We observed that the expression level of HILS1 transcript was significantly decreased in Sertoli cell only syndrome and complete maturation arrest at second spermatocyte in comparison to the positive control. We also found some microRNAs, particularly miR-363, potentially regulating HILS1 transcript at the post-

transcriptional level. Moreover, our analyses suggested that HILS1 might be processed into microRNAs homologous to miR-5588 and miR-665.
Conclusion: Our collective results suggest that regulation of HILS1 might play an important role in human spermiogenesis, which may have implications for improved treatment of men's infertility.
Key words: *HILS1, Spermiogenesis, Infertility, MicroRNA, Bioinformatics.*

P-158
Investigation of epigenetic factors involved in male infertility

Hadi N[1], Namazi F[1], Nateghi B[2].
1.*Department of Genetic, Islamic Azad University, Ashkezar Branch, Ashkezar, Iran.*
2.*Department of Biochemistry, Faculty of Science, Nourdanesh Institutions of Higher Learning, Meimeh, Isfahan, Iran.*
Email: behnaz.natrqi@gmail.com

Background: About 8% of men are treated for infertility problems. Several factors are involved in male infertility, and in general, genetical, epigenetically and non-genetically among the most important factors. The investigation of mitochondrial DNA and diversity of RNAs is one of the genetic causes of male infertility. Several studies have shown that the source of many infertile factors in men is the mitochondrial DNA and RNAs. The most important epigenetic causes associated with males' infertility include DNA methylation, genetic marking, and histone changes after translation. In the current study, the diversity of RNAs in sperm, the relationship of males' infertility with miRNAs, epigenetic changes including DNA methylation, genetic marking, and histone changes after translation have been described.
Objective: In the current study, the diversity of RNAs in sperm, the relationship of males' infertility with miRNAs, epigenetic changes including DNA methylation, genetic marking, and histone changes after translation have been described.
Materials and Methods: The present study is an overview based on information collected from published research related to the topic.
Results: The role of RNAs in male infertility has been identified, as well as epigenetic signals from the sperm genome are important in the performance of sperms so that any change in this process can change the performance of the sperm.
Conclusion: It clear that these factors should be considered as an effective factor in infertility.
Key words: *Male infertility, Epigenetic factors, RNA.*

P-159
Ultrasensitive biosensor for breast cancer screening

Hakimian F[1], Ghourchian H[1], Haghiralsadat B[2].
1.*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.*
2.*Department of Advanced Medical Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: ghourchian@u.ac.ir

Background: MicroRNAs (miRNAs) are non-coding and single-stranded RNAs between 17-22 nucleotides that found in plants, animals and some viruses. Expression of miRNAs is changed in tumor compared to normal tissue and can be used as biomarkers for early detection of cancer diseases. Previous studies indicated that miRNA-155 plays an important role in the pathogenesis of breast cancer. Since the expression of miRNA-155 rises in patients with breast cancer, so it is considered as a breast cancer risk factor.
Objective: We introduced an electrochemical biosensor for miRNA-155 detection with high sensitivity for breast cancer screening.
Materials and Methods: Briefly, probe was immobilized through self-assembly onto a gold electrode. Then, target (miRNA-155) was hybridized to the probe. In the final step, silver nanoparticles were casted onto the modified gold electrode. Due to the oxidation of silver nanoparticles, significant electrochemical signal is produced.
Results: The designed electrochemical biosensor provided an ultrasensitive detection of miRNA-155 at very low concentrations with the detection limit of 20 zeptomoles and a wide linear range from 20 to 2×109 zeptomoles. Moreover, such biosensor detected miRNA-155 in serum samples with satisfactory results.
Conclusion: In the current work, a versatile, simple, cost-effective and highly sensitive electrochemical biosensor for the detection of miR-155 is presented. This sensor achieves an ultralow detection limit. Moreover, normal and cancerous serums were diagnosed by the biosensor successfully that is important for biomedical research and clinical POC applications.
Key words: *Silver nanoparticles, miRNA-155, Breast cancer, Biosensor.*

P-160
The importance of amnioMAX medium in the recovery of human amniotic fluid cells from freezing at -80°C

Hoseini SM[1], Montazeri F[2], Karimi R[2], Heidarian Meimandi H[2], Ghasemi-Esmailabad S[2], Kalantar SM[2].
1.*Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical, Yazd, Iran.*
Email: smkalantar@yahoo.com

Background: Recent developments in regenerative medicine led to a rapid rise in the use of stem cells and strategies for preservation and storage. Therefore, preparing a frozen stock of cells properly increases the validity of any results generated from the cell line.
Objective: This paper examines the effect of medium on retrieving of human amniocytes from freezing at -80°C.
Materials and Methods: Amniocytes were cultured in DMEM medium and then cells were frozen at passage 4 in FBS supplemented by 10% DMSO under the following conditions: 10 min at 4°C, 24 hr at -20°C and

then cryopreserved in -80°C in four different time range including 4 months, 6 months, 8 months and 1 year, each group includes 3 samples. For recovery of the amniocytes, we used 3 protocols: AmnioMAX-II, DMEM and a modified medium composed of 2:1 v/v DMEM: AmnioMAX-II.

Results: Our results have shown total recovery by three protocols in the group 4m. While in 6m group, only 2 samples in DMEM group could not be able to grow. However, the cells demonstrated incredibly lower viability in the time course of 8m and 1y, so that in the group DMEM only 1 sample in 8m and none of them in 1y category was able to proliferate. Nonetheless, this was amazingly different when we used AmnioMAX, as a results, recovery with AmnioMAX was 100% in all samples, from 4m to 1y of cryopreservation, even though DMEM:AmnioMAX-II (2:1 v/v) could recover only 2 samples in 8m and one sample in 1y.

Conclusion: Our work has led us to conclude the importance of AmnioMAX medium in the recovery of human amniocytes in the long-term course of freezing at -80°C.

Key words: *Amniotic fluid cells, Freezing procedure, AmnioMAX, Cryopreservation.*

P-161

Methylenetetrahydrofolate reductase C677T polymorphism and its association with recurrent pregnancy loss in South-Eastern Iranian population

Hoseini SM[1, 2], Montazeri F[3], Hajimaghsoudi E[1, 2], Zarein F[3], Rahmani S[1, 2], Sheikhha MH[1].

1.*Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Khatam Al-anbia Superspecialty Clinic, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: sheikhha@yaho.com

Background: An increasing number of studies have found that inherited thrombophilia can be associated with Recurrent Pregnancy Loss (RPL) in various populations all over the world. In this regard, one of the most studied polymorphisms is Methylenetetrahydrofolate Reductase (*MTHFR*) C677T which is suggested to be a prevalent etiology of RPL.

Objective: This study aimed to investigate the association between C677T mutation and RPL in 370 women with idiopathic RPL and 220 women without history of abortion and at least 1 term pregnancy as the control group.

Materials and Methods: Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen). To identify C677T mutation, we performed the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method. The frequency of mutation was statistically compared between two groups to examine whether significant differences exist between them.

Results: Our results have shown the frequency of 24.9% in C677T mutation (heterozygote and homozygote) which was significantly higher in patients ($p<0.05$).

Conclusion: As expected depending on the results of same investigation in other populations, *MTHFR* C677T mutation is responsible for a considerable increase in the risk of RPL.

Key words: *C677T mutation, ARMS-PCR, MTHFR, RPL.*

P-162

Mitotic arrest deficient 1 like 1 (MAD1L1) gene variations in products of conception with aneuploidy

Hosseini N[1, 2], Kariminejad R[3], Amiri Yekta A[2], Kariminejad A[3], Najmabadi H[3], Bazrgar M[2].

1.*Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran.*
2.*Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3.*Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran.*

Email: nargeshosseini.gene@gmail.com; mbazrgar@royaninstitute.org

Background: More than 57% of spontaneous abortions are due to chromosomal abnormalities, certainly aneuploidies. Although aneuploidy is associated with advanced maternal age it is frequent in young women. Spindle assembly checkpoint (SAC) complex has a critical role in correct chromosome segregation during cell division. Several genes are involved in SAC, one of them is MAD1L1.

Objective: We investigated pathogenic single nucleotide variations (SNVs) of MAD1L1, rs121908982 and rs121908981 and their upstream and downstream SNVs to investigate their association with aneuploidy in products of conception (POC).

Materials and Methods: POC of mothers aged <36 were analyzed using quantitative fluorescence polymerase chain reaction (QF-PCR) and/or array comparative genomic hybridization (aCGH). Those with aneuploidy were enrolled in genotyping. Areas of interest of MAD1L1 were genotyped using PCR followed by Sanger sequencing. Comparison of the observed SNVs frequencies with the highest population minor allele frequency (MAF) was performed using Chi Square.

Results: According to QF-PCR and aCGH results, 40 aneuploid samples enrolled in genotyping. We didn't observe those pathogenic SNVs but observed rs10260386, rs1481591257, rs10257349, rs372373978, rs752408355, rs1639921, rs376061507, rs62442486, rs74431414 SNVs with following allele frequencies and p-values of comparison with the highest population MAF: A:0.7/G:0.3 ($p<0.0001$), G:0.9875/A:0.0125 ($p=0.8222$), T:0.4/C:0.6 ($p=0.2816$), G:0.9875/0.0125 ($p=0.8222$), A:0.9875/G: 0.0125 ($p=0.1775$), T:0.875/C:0.125 ($p<0.0001$), T:0.6806/C:0.3194 ($p<0.0001$), A:0.68/G:0.32 ($p<0.0001$) and G:0.9583/T:0.0417 ($p=0.9425$), respectively.

Conclusion: Frequencies of rs10260386, rs1639921, rs376061507 and rs62442486 SNVs were obviously

higher than the highest population MAF. These SNVs seem to be associated with aneuploidy in POC.

Key words: *Abortion, Aneuploidy, MAD1L1, Single nucleotide variation (SNV).*

P-163

Investigation of changes in expression level of *XRCC2* gene in obstructive azoospermia and nonobstructive azoospermia patients

Hosseinnia M[1], Babakhanzadeh E[2], Talebi M[2], Khodadadian A[2], Ghovvati S[1].

1.*Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran.*
2.*Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: ghovvati@yahoo.co.uk

Background: Infertility is one of the major problems in the world. Identifying genes that play a biomarker and diagnostic role can be very important. X-ray Repair Cross Complementing 2 (*XRCC2*) gene is involved in the homologous recombination repair (HRR) pathway of double-stranded DNA, thought to repair chromosomal fragmentation, translocations and deletions, and plays an essential role in gametogenesis. A *XRCC2* recessive mutation causing infertility in human. Also knockout of Xrcc2 in mice has shown embryonic lethality, meiotic arrest and infertility in male.

Objective: The aim of this experiment was to evaluate variation in *XRCC2* gene expression in OA and NOA patients.

Materials and Methods: In the present study, we aim to evaluate expression levels of the *XRCC2* gene, in the testicular biopsy samples obtained from men with various types of NOA including obstructive azoospermia (OA, n=8), maturation arrest (MA, n=10), Sertoli cell only syndrome (SCOS, n=11) and hypospermatogenesis (HP, 9). The relative expression of *XRCC2* gene was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Kruskal-Wallis test was used to determine inter-group differences in *XRCC2* gene expression among the four histologic groups.

Results: Examination consists of the detection of mRNA uncovered a severe decrease of XRCC2 in samples with MA and HP compared with samples with OA ($p<0.05$).

Conclusion: The finding of this study shows that XRCC2 has an expression in the germ cells in the spermatocyte stage and possibly in meiosis phase and post- meiotic stages ($p<0.05$). This result suggesting that a deficient expression of XRCC2 might be reflecting and/or contributing to round spermatid maturation arrest. And in order for these statements to be finalized, there is a need for protein measurements and more samples.

Key words: *XRCC2, Obstructive azoospermia, nonobstructive azoospermia.*

P-164

XO expression among healthy vs. oligospermia affected men before and after treatment with the palm pollen: in vivo research

Izadi Raeini M[1], Allameh Zadeh Z[2], Fallahi S[3], Malekzadeh K[3], Izadi Raeini M[4].

1.*Department of Genetic, College of Science, Islamic Azad University, Ashkezar Branch, Yazd, Iran.*
2.*Department of Biology, College of Science, Islamic Azad University, Arsanjan Branch, Shiraz, Iran.*
3.*Fertility and Infertility Research Center, Faculty of Medical Sciences, Bandarabbas University of Medical Sciences, Bandarabbas, Iran.*
4.*Department of Chemistry, College of Sciences, Payame Nour University, Sirjan, Iran.*

Email: maliheizadi97@yahoo.com

Background: Exclusive cause of about 20% of couple infertilities is due to male abnormalities. In other 20-40% of the cases, the same issue is still a crucial role-player. On the other hand, oxidative-stress is considered as an important factor, deriving male infertility. There are confirmed evidence on phoenix dactylifera pollen (DPP) antioxidant properties, which are able to improve sperm quality. Xantin oxidase (XO) catalyzes xantin and hypoxantin oxidation in porin metabolism. This anzyme decreases molecular oxygen and culminate to a formation superoxide. Which will then play a role in sperm production through ROS.

Objective: The objective of this study is to evaluate the effect of DPP on sperm parameters and expression of XO.

Materials and Methods: 40 oligospermia affected and 10 healthy male individuals were studied. The affected group were treated for 40 consecutive days, Using gelatin capsules with 4 g DPP. Semen samples were obtained from all subjects both before and after the treatment. To assess sperm parameters, free 8-Isoprostane with Elisa method was tested as an index of DNA damage. mRNA expression of XO gene with real-time PCR method was tested in both stages. Data studied with statistical software SPSS.

Results: XO expression was higher among the oligospermia affected group than the healthy control group. Infertile men treated with DPP had a significant reduction in the XO mRNA expression levels ($p<0.05$). A similar reduction was also observed in free 8-Isoprostane. Besides, sperm parameters were improved ($p<0.05$).

Conclusion: These findings revealed that oxidative stress was truly a key factor involved in male infertility. DPP showed a positive effect on fertility factors and reducing sperm oxidative stress-related lethality. This herbal therapeutic agent can therefore be used as a low-risk therapeutic approach in treatment of male infertility.

Key words: *Infertility, Oligospermia, DPP, XO.*

P-165

Determination of Chlamydia trachomatis incidence and its role on recurrent pregnancy loss (RPL)

Kafi S[1], Ghasemi N[1], Dehghan Marvast L[2], Kimiaee Sadr M[3], Aflatoonian A[2].

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

2.*Research and Clinical Center for Infertility, Yazd Reproduction Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Microbiology, Islamic Azad University, Falavarjan Branch, Isfahan, Iran.*
Email: abbas_aflatoonian@yahoo.com

Background: Chlamydia trachomatis (C.T) is an obligately intracellular bacterium that is responsible for an increasing number of sexually transmitted infections in both males and females. C.T infection is the most common sexually transmitted disease (STD) in the world that can persist and also ascend in the genital tract. This intracellular and silent infection is related to some adverse pregnancy outcomes, such as miscarriage.
Objective: The aim of the study was to evaluate the frequency of C.T infection among women who experienced a miscarriage.
Materials and Methods: 80 female (age 17-40 yr) partners of infertile couples took pair in this study when seeking for treatment in Yazd Research and Clinical Center for Reproductive. After obtaining informed consent, women with history of ≥2 miscarriages entered the study. To achieve the objectives of this study, PCR were performed on vaginal swabs samples.
Results: There was three positive sample using PCR.
Conclusion: The results of this study showed that CT infection was not common among patients in the study population. However, as the population under study was not representative of Iranian women, therefore the study needs larger population for decision.
Key words: *Chlamydia trachomatis, Miscarriage, Recurrent pregnancy loss.*

P-166
Overexpression of *CREB* gene in endometriotic biopsies of women with endometriosis

Kalanatari S[1], Saadat Varnosfaderani A[2], Ramezanali F[3], Shahhoseini M[2], Amirchaghmaghi E[4].
1.*Department of Cell and Molecular Biology Science, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran.*
2.*Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute, for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
4.*Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
Email: shahhoseini244@gmail.com

Background: Endometriosis is an estrogen-dependent disease that affects women in reproductive age. In this disease, the endometrial tissue is implanted outside the uterine cavity, mainly in the pelvic area. Endometriosis is associated with pelvic pain and infertility. Aberrant expression of enzymes involved in biosynthesis of estrogen, steroidogenic acute regulatory protein (StAR) and aromatase (rate limiting enzyme in estrogen biosynthesis), have been reported in the endometrium of women with endometriosis. In addition, prostaglandin E2 (PGE2) is a potent inducer of StAR and aromatase gene expression in endometrial stromal cells and impresses its effect via cAMP response element binding protein (CREB). CREB is a member of basic leucine zipper transcription factors family that has binding site on the promoter of StAR and aromatase genes. On the other hand, it has been shown that overexpression of CREB is associated with cell proliferation, apoptosis inhibition and cell invasion.
Objective: The goal of this study was to evaluate the gene expression of CREB in eutopic and ectopic endometrial tissues of women with endometriosis compared with control endometrial samples.
Materials and Methods: Ectopic and eutopic endometrial tissues were obtained from 10 women with endometriosis (endometriosis group). The diagnosis of endometriosis was laparoscopically approved. Control tissue samples were collected from 10 women who had no evidence of endometriosis during laparoscopy (control group). All studied women had no history of hormonal treatment before endometrial sampling. After endometrial tissue collection, RNA extraction and cDNA synthesis were performed for both groups. The real-time PCR technique was used to examine the gene expression of CREB. The results were analyzed using one way ANOVA as statistical method.
Results: Gene expression of CREB was significantly higher in ectopic endometrium of women with endometriosis than control and eutopic endometrial samples ($p<0.05$), while its gene expression was approximately the same in control and eutopic endometrial tissues.
Conclusion: The increased gene expression of CREB in ectopic lesion of endometriosis may contribute in its pathogenesis via cell proliferation, apoptosis inhibition and cell invasion.
Key words: *Endometriosis, CREB, Ectopic, Eutopic, Endometrium.*

P-167
Mir-147 act as a tumor suppressor in ovarian cancer with targeting the *ZEB1* and *ZEB2* genes

Khodadadian A, Babakhanzadeh E, Mazaheri M.
Department of Medical Genetics, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: mahta.mazaheri2019@gmail.com

Background: In women, ovarian cancer has an important role in mortality due to cancer. This malignancy is the eighth most common cancer among Iranian women. Surgery and chemotherapy are common treatments for cancers, including ovarian cancer. Most ovarian cancers are epithelial. The main problem with this type of malignancy is the difficulty in detecting it.
Objective: In this study, we aimed to evaluate the expression of ZEB-1, ZEB-2, and Mir-147 in the ovarian cancer cell line before and after treatment with cisplatin as well as ovarian cancer tissues.
Materials and Methods: In this study, 30 ovarian cancer specimens coupled their tumor marginal tissue were collected from Shahid Sadoughi Hospital. Sensitive

(A2780S) and resistant (A2780CP) cell lines of ovarian cancer were grown in RPMI culture media. Then, cells were treated with different concentrations of cisplatin and cisplatin-IC50 was measured by MTT assay. RNA was extracted from treated cells and tissues and after cDNA synthesis, qRT-PCR was performed. Finally, the results were analyzed.

Results: In 23 tumor samples, expression of Mir-147 showed a significant reduction in expression than controls ($p=0.0001$). The expression of ZEB-1and ZEB-2 genes expression in these tumor samples was significantly higher than controls ($p<0.001$). In the cisplatin-sensitive cell line, the expression of Mir-147 increased significantly after 24, 48 and 72 hr treatments ($p<0.05$). Also, a significant increase was observed in the resistant cell line after 72 hours of treatment with cisplatin. After 24, 48 and 72 hr cisplatin treatment, there was a significant decrease in expression of ZEB-1 and ZEB-2 genes in sensitive cell line. Moreover, 72 hr after treatment, there was a decrease in the expression of these genes in the resistant cell line.

Conclusion: The results of this study showed a reduction in the expression of Mir-147 and an increase in the expression of the ZEB-1 and ZEB-2 genes in tumor tissue relative to its marginal control tissue. It also increased the expression of Mir-147 in the cell line after treatment with cisplatin and decreased expression of the ZEB-1 and ZEB-2 genes. Therefore, it can generally be concluded that in the tissue samples and ovarian cancer cell lines, the expression of Mir-147 as a tumor suppressor and ZEB-1 and ZEB-2 genes as an oncogene has been impaired.

Key words: *Ovarian cancer, MIr-147, Gene expression, Cisplatin treatment.*

P-168
Detection of 4977-bp deletion of mitochondrial DNA in in vitro fertilization failure women

Mirabutalebi SHR [1, 2], Karami N[1], Ashrafzadeh HR[3], Akhavansales Zh[4], Tavakoli M[5], Ghasemi N[3].

1.*Genetics Department, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
5.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: hr_mirabutalebi@yahoo.com

Background: The quality of oocyte is often considered as a limiting factor for fertility, especially IVF. Some mitochondrial mutations, particularly the 4977-bp deletion increase with the age. Thus, this mutation can serve as a marker for cell aging, which indicates the reduced quality of the oocytes for fertilization. It has been suggested that this can also be investigated in the blood cells of women with IVF failure.

Objective: 1-Determination of the frequency of 4977-bp deletion in women with IVF failure, 2-Investigation of the relationship between 4977-bp deletion and the age of patients.

Materials and Methods: Polymerase chain reaction was used to detect the 4977-bp deletion in blood samples of 52 IVF failure women and 52 women who had at least one healthy child. After polymerase chain reaction with deleted and wild-type primers, the products were examined using agarose gel electrophoresis.

Results: 48.07% of women with IVF failure and 34.62% of healthy women had a mitochondrial 4977-bp deletion, with $p=0.163$ and OR: 1.749. Also, in association with the age of these patients and the frequency of 4977-bp mutation, p and OR were obtained 0.163 and 1.749, respectively and frequency of this mutation was higher in patients over 35 yr old compared to other subgroups (Patients $\geq$35: 57.69).

Conclusion: According to the findings of this study, there is no a significant relationship between the frequency of mitochondrial 4977-bp mutation and failure in IVF.

Key words: *In vitro fertilization, Mitochondria, Mutation, Cell aging.*

P-169
Evaluation of QF-PCR in simultaneous detection of trisomy 21 and monogenic diseases in PGD

Mirmohammadvali[1] S, Reihani Kermanshahi F[2], Eftekhari Yazdi P[3], Gourabi H[2], Saffari J[1], Dalman A[3], Zareei M[1], Bolouri S[2], Babaabasi B[2], Zamanian M[2].

1.*Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Central Tehran Branch, Tehran, Iran.*
2.*Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
3.*Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

Email: zamanzss@gmail.com

Background: Trisomy 21 (Down syndrome) is occurring in one of 700-800 live births. Preimplantation genetic screening (PGS) is a method used in women with recurrent abortions, frequent ART failures and older age pregnancies enabling selection of normal chromosomal embryos which prepares for healthy successful pregnancies. Currently, various methods are used for PGS including FISH, aCGH and qPCR while each one has its own advantages as well as disadvantages. QF-PCR which is extensively used for the prenatal screening of the common aneuploidies can be recommended as a reliable alternative method for PGS due to its relative advantages including speed, low cost and high sensitivity/specificity. In addition, it can be used on embryo for the concurrent detection of single gene disorders through single blastomere biopsy.

Objective: Present study was aimed to evaluate the capability of QF-PCR in screening for trisomy 21 on single blastomere biopsies.
Materials and Methods: Four STR markers were used to evaluate the ploidy of chromosome 21 using DNA from whole genome amplification (WGA) of single blastomeres from 30 trisomic/disomic embryos. The reactions were optimized in advance using single lymphocytes while the STR markers were also checked for desired heterozygosity in Iranian population.
Results: QF-PCR showed a relative capability to screen for trisomy 21 on embryonic samples.
Conclusion: Although QF-PCR could be applied for the screening of chromosome 21 trisomy in embryo, due to some limitations including allele drop out (ADO), preferential amplification (PA) and non-specific signals; it will be necessary to take measures for further optimization of the reactions in a larger sample size. In addition, using nested PCR instead of DNA from WGA may decrease the rates of ADO and PA. Finally, the test should be checked in terms of its sensitivity and specificity to make it applicable for clinical application.
Key words: *PGD, Trisomy 21, SGD, QF-PCR.*

P-170
The effect of preincubation time on oxidative stress and mitochondrial alteration of mouse MII oocytes in the simple and myo-inositol supplemented media

Mohammadi F[1], Ashrafi M[2], Zandieh Z[1], Najafi M[3], Niknafs B[4], Amjadi FS[1], Haghighi M[1].
1.*Department of Anatomy, Iran University of Medical Sciences, Tehran, Iran.*
2.*Shahid Akbar Abadi Clinical Research Development Unit, Iran University of Medical Sciences, Tehran, Iran.*
3.*Department of Biochemistry, Iran University of Medical Sciences, Tehran, Iran.*
4.*Department of Anatomy, Tabriz University of Medical Sciences, Tabriz, Iran.*
Email: Ashrafi.m@iums.ac.ir

Background: Preincubation is the temporary cultivation of oocytes at 37°C and 5-6% of CO_2 before ART procedures. There is not any explanation regarding a standard preincubation time in ART laboratory guidelines and it is dependent completely on the laboratory workload. Myo-inositol as the most important form of inositol, is involved in several systemic processes and its antioxidant action has been suggested recently.
Objective: The study aimed to evaluate the effect of preincubation time of mouse MII oocytes in the simple and myo-inositol supplemented medium on the oxidative stress and mitochondrial alterations.
Materials and Methods: Cumulus Oocyte Complexes which were retrieved from superovulated NMRI mices were divided randomly in five experimental groups: (1) control (2) 4 hours preincubation in simple medium (3) 4 hours preincubation in 20 mmol/l of myo-inositol supplemented medium (4) 8 hours preincubation in simple medium (5) 8 hours preincubation in 20 mmol/l of myo-inositol supplemented medium. COCs were denuded and intracellular Reactive Oxygen Species (ROS), glutathione (GSH), Mitochondrial Membrane Potential (MMP) and mitochondrial distribution were measured by a fluorometric assay. ATP content of oocytes also was measured using the ELISA method.
Results: The ROS and GSH levels, mitochondrial distribution, MMP as well as ATP content were significantly different between groups. Levels of ROS, GSH and mitochondrial distribution were improved in oocytes preincubated in MYO supplemented medium compared to simple medium. The groups with 4 hours preincubation in simple media and 8 hours preincubation in supplemented media were at the same level in terms of the ROS, GSH and mitochondrial distribution. MMP of oocytes was not reduced after 4 hr of preincubation, but 8 hr of preincubation time could decrease it significantly. ATP content did not decline in oocytes preincubated for 4 and 8 hr, while supplementation of MYO could increase it in groups with 4 and 8 hr of preincubation.
Conclusion: Short preincubation time can influence the oocyte quality related to alternation in ROS, GSH, MMP and mitochondrial integrity. Supplementation of MYO in mediums improves the quality drop significantly in addition to groups with preincubation in the simple media but does not prevent quality drop compared to the control group.
Key words: *Oocyte preincubation time 1, Oocyte quality 2, Myo-inositol supplement 3, Oxidative stress 4, Mitochondrial alteration 5.*

P-171
mRNA level of *FSHR* gene as a biomarker in PCOs patients

Montazeri F[1], Fesahat F[2], Hoseini SM[3], Kalantar SM[1], Sheikhha MH[3].
1.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: sheikhha@yahoo.com

Background: Selecting good quality oocyte and embryo with high implantation potential is of great importance in assisted reproductive technologies (ART). To date, the choice of the best oocyte and embryo for transfer have been based on morphological parameters. Therefore, movement towards modern technologies such as genomics, transcriptomics, proteomics and metabolomics to select the most competent oocytes and/or embryos with the greatest implantation potential would be significant.
Objective: We aimed to assess the *FSHR* gene expression of cumulus cells (CCs) between mature and immature oocyte in order to evaluate oocyte maturity in PCOs patient.
Materials and Methods: The CCs were retrieved from 30 PCO women and divided into groups of GV and MII

according to the nuclear maturity status in order to studying expression patterns of *FSHR* gene using Q-PCR.
Results: There were significant differences ($p<0.05$) in mRNA levels of *FSHR* gene between the groups.
Conclusion: It appears the expression level of these genes in cumulus cells have the potential to be a predictive non-invasive biomarker for oocyte maturity and quality.
Key words: *Polycystic Ovarian Syndrome (PCOs), Oocyte maturity, Embryo selection, FSHR gene, Biomarker.*

P-172
Diagnostic value of FTS and QUAD tests in detecting chromosomal abnormalities compared to FISH and KARYO tests (as gold standard)

Montazeri F[1], Razavi D[2], Sabbagh Nejad Yazd S[1], Heidarian Meimandi H[1], Ghasemi-Esmailabad S[1], Kalantar SM[1].
1.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
2.*Department of Biology, Sciences Faculty, Science and Arts University, Yazd, Iran.*
Email: smkyzd@gmail.com

Background: Congenital anomalies such as Down syndrome and other chromosomal aneuploidy in 13, 18, 21 and XY chromosomes have many economic, social, cultural and psychological burdens for families and society. Therefore, early diagnosis in the first trimester could prevent the complications of these abnormalities. For confirmation of screening tests, invasive tests are used**.**
Objective: In order to compare the results of screening and invasive tests and to determine the degree of effectiveness this study was conducted.
Materials and Methods: The medical record of 970 pregnant women referred to the Yazd Reproductive Science Institute was investigated in this study. Finally, 367 cases included which were the pregnant women under 35 yrs. old at 18 to 10 weeks of gestation. The screening results of patients who performed first and second trimester screening tests were compared. On the other hand, screening results compared with the results of invasive diagnostic tests include FISH and Karyotype. Then, statistical analyzing of data by alpha software and Q-square test were performed, diagnostic power and accuracy of the first and second trimester screening tests were determined. The analysis was performed using SPSS software version 20. The significance level was considered $p<0.05$.
Results: The results of the comparison of screening tests with confirmatory tests showed that all screening tests for the first trimester and the second trimester were trisomy 21 screening with a sensitivity of 90% up to high, but unfortunately, the lower prevalence was in the range of 23% to 62% They can be calculated, which indicates that the false positive reports of these tests are high.
Conclusion: Combined screening test with a sensitivity of 100% and specificity of 92% can be safely used up to prevent high-risk pregnancies. Therefore, it can be concluded that in order to obtain a better result with a higher degree of confidence, both the first and second quarterly screening tests should be carried out sequentially. We hope that eventually, improving screening tests can reduce the number of referrals for invasive diagnosis tests and their associated risk of abortion.
Key words: *Amniocentesis, Screening test, Invasive test, Karyotype, FISH.*

P-173
Isolation, characterization and multilineage differentiation potential of Wharton's jelly mesenchymal stem cells

Moshrefi M[1, 2], Salehinejad P[3], Seyedi F[3, 4], Nasiri E[3, 5], Nematollahi-mahani SN[3].
1.*Medical Nanotechnology and Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Anatomy, Kerman University of Medical Sciences, Kerman, Iran.*
4.*Department of Anatomy, Jiroft University of Medical Sciences, Kerman, Iran.*
5.*Campus of Paradise, Islamic Azad University of Shiraz, Shiraz, Iran.*
Email: nnematollahi@kmu.ac.ir

Background: Wharton's jelly, as the matrix of umbilical cord, contains two major populations of cells including fibroblast like cells and primitive stromal cells. The majority of these primitive stromal cells are fibroblast like while the others demonstrate round shape. They express F-actin protein and can be aggregated as the colony like structures which express alkaline phosphatase. These primitive stromal cells display the characteristics of mesenchymal stem cells, as they have great expansion capacity and express mesenchymal markers like CD44, CD73, CD90 and CD105. But, the expression of CD34 and CD45 is negative in these cells. In addition, osteogenic and adipogenic induction of these cells results in differentiation into osteoblast and adipocyte cells.
Objective: In our experiments we analyzed the multi lineage differentiation of these cells.
Materials and Methods: Parallel to other researches, in our studies we have observed that under the induction media they can differentiate toward special cell lineage. For example, in our studies we observed that under the induction of retinoic acid, dimethyl sulfoxide, fibroblast growth factor and epidermal growth factor, these cells can differentiate to the cells of neural system and the differentiated cells express the neural stem/progenitor cell marker nestin. Also, we concluded that retinoic acid was the main inducer for neuron and oligodendrocyte formation while for astrocyte differentiation, DMSO was crucial. Beside the several previous studies for pancreatic islet like differentiation, in our trial we proposed a new

modified induction protocol together with hanging drop culture system which led to the formation of pancreatic islet like cells and production of significantly higher amounts of insulin and proinsulin than the other most common protocols. Another organized study was on Wharton's jelly mesenchymal stem cells differentiation into cardiomyocytes like cells. In this study, under the induction of different cocktails including 5-azacytidine, ascorbic acid, hypoxia and conditioned medium, derived from neonatal heart cell culture, we observed that the umbilical cord mesenchymal stem cells exhibited cardiac cell morphology and expressed GATA, conexin43, alpha actin and cardiac troponin.

Results: Differentiation of these cells to other kinds of cells including hepatocyte like cells, bone, fat and muscle has been reported, repeatedly. Altogether, on the bases of our results we affirm the mesenchymal nature and in vitro multilineage differentiation capacity of Wharton's jelly mesenchymal stem cells.

Conclusion: Previously, the idea was that, Wharton's jelly mesenchymal stem cells exhibit multipotent differentiation capacity and can differentiate into several cellular lineages. But since there are several reports regarding their differentiation to germ like cells as well, we can propose these cells as pluripotent stem cells. Therefore, differentiation capacity into multiple tissue cells makes them a good candidate with various abilities for cell therapy procedures.

Key words: *Wharton's Jelly, Mesenchymal Stem, Multilineage Differentiation.*

P-174

Association of the folate metabolizing pathway polymorphisms with male infertility susceptibility

Moudi M.

Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

Email: mahdiyehmodi@yahoo.com

Background: Infertility is a global health dilemma and a multifactorial disorder affecting approximately 10-15% of all couples. It has been shown that polymorphic variants of the genes encoding key enzymes of folate and methionine metabolism may influence DNA methylation. Methylene tetra hydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) are three key enzymes of the homocysteine and folate metabolic pathways.

Objective: Polymorphisms of the folate metabolizing pathway were associated with male infertility susceptibility.

Materials and Methods: The data were extracted from published articles about the polymorphisms of MTHFR pathways and the infertility from 2000 to 2018. The polymorphisms of MTHFR rs1801133 C>T, MTR rs1805087 A>G, MTHFR rs1801131 A>C and MTRR rs1801394 were selected.

Results: The MTHFR C677T mutation was reported a risk factor for male infertility in both azoospermia and oligoasthenoteratozoospermia patients, especially in Asian population. The MTHFR TC haplotype were determined as high risk in men infertility while men carring CC haplotype had lowest risk. On the other hand, the MTHFR A1298C mutation was not associated to male infertility. MTR A2756G and MTRR A66G were potential candidates in the pathogenesis of male infertility, but more case-control studies were required to avoid false-positive outcomes.

Conclusion: In conclusion, the findings indicated that the genetic mutations in the folate-related enzyme genes played a significant role in male infertility.

Key words: *Infertility, Folate Metabolizing Pathway, MTHFR rs1801131 A>C and MTHFR rs1801133 C>T, MTR rs1805087 A>G, MTRR rs1801394.*

P-175

Association between G1691A factor V Leiden mutation and idiopathic RPL in the Iranian population of Yazd province

Rahmani S[1, 2], Hoseini SM[1], Montazeri F[3], Zarein F[3], Hajimaghsoudi E [1], Sheikhha MH[1].

1. *Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2. *Khatam Al-anbia Superspecialty Clinic, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3. *Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences Services, Yazd, Iran.*

Email: sheikhha@yahoo.com

Background: Factor V Leiden G1691A gene mutation is an important cause of thrombophilia, the condition is related to recurrent pregnancy loss (RPL). RPL is defined as two or more consecutive miscarriage before 20 wk of gestation, and can be caused by genetic factors including parental chromosomal abnormalities and thrombophilia as well as environmental factors such as uterine anomalies.

Objective: The present paper aims to investigate the association of Factor V Leiden mutation in women suffered from RPL and women without history of abortion and at least 1 term pregnancy as the control group in Yazd province of Iran.

Materials and Methods: Genomic DNA was extracted from peripheral blood of 370 patient with using QIAamp DNA Blood Mini Kit (Qiagen). We studied 370 cases and 160 controls to investigate the potential association between G1691A and RPL. In this study, samples were collected between 2015 and 2019 in Yazd Genome Genetics Laboratory.

Results: The frequencies of Factor V Leiden G1691A mutation (heterozygote, normal homozygote and mutant homozygote) were 4.4%, 95.4% and 0.3% ($p<0.05$) in cases, respectively.

Conclusion: The results indicated a significant higher frequency of Factor V leiden G1691A in women with RPL in comparison with controls. Accordingly, the G1691 could be used as a genetic marker for early diagnosis of RPL.

Key words: *RPL, Factor V leiden, G1691A, FVL.*

P-176

Assessment of the circulating mir-218 expression level in under treatment epileptic patients as a noninvasive biomarker of PCOs

Rajabi M[1], Montazeri F[1], Mirsmaeili SM[2], Salehi M[3], Etemadifar M[4], Kalantar SM[1].

1.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Biology, Sciences and Art University, Yazd, Iran.*
3.*Department of Medical Genetic, Isfahan University of Medical Sciences, Isfahan, Iran.*
4.*Department of Neurology, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: smkalantar@yahoo.com

Background: Epilepsy is a common chronic neurologic condition with unknown mechanism. Moreover, side effects of antiepileptic drugs specially valproic acid (VPA) which is commonly used the treatment of epilepsy; is of interest. In women, VPA can also lead to androgenization, menstrual disturbances and PCOs. The incidence of polycystic ovary syndrome (PCO) increases in women with epilepsy (WWE).

Objective: We aimed to define a non-invasive biomarker for early diagnosis of PCOs in WWE patients and to take preventative measures.

Materials and Methods: The study was conducted on 15 WWE women at reproductive ages (20-40 yr) with PCOs, monotherapy with VPA and 15 women without PCOs. The women with history of diabetes, kidney and liver disease was excluded. After collecting patient 'serum, total RNA were extracted. We use Q-RTPCR to identify the expressions level of miRNA-218 in epileptic patients with PCOs and without it.

Results: In accordance with previous studies on PCOs and non-PCOs women, expressions level of miRNA-218 were significantly different between epileptic patients with pco and without it ($p<0.05$).

Conclusion: Our results suggesting miRNA-218 as non-invasive biomarker to screen for drug side effects and prevent the progression of its complications. Furthermore, bioinformatics analysis indicate role miRNA-218 in Estrogen/ Adiponectin/ MAPK signaling pathway which may be of importance in PCOs.

Key words: *Epilepsy, PCO, Non-invasive biomarker, miRNA-218.*

P-177

Evaluating expression level of the circulating mir-146 in under treatment epileptic patients as a noninvasive biomarker of PCOs

Rajabi M[1], Montazeri F[1], Hosseini S[1], Etemadifar M[2], Salehi M[3], Kalantar SM[1].

1.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Neurology, Isfahan University of Medical Sciences, Isfahan, Iran.*
3.*Department of Medical Genetics, Isfahan University of Medical Sciences, Isfahan, Iran.*

Email: smkalantar@yahoo.com

Background: Epilepsy is a common chronic neurological disorder affecting approximately 50,000,000 people by recurrent unprovoked seizures. However, there is increasing concern that the pathogenic epilepsy mechanism remains poorly defined. Antiepileptic drugs are known to have endocrine side effects. Valproic acid (VPA) is widespread drug in the treatment of epilepsy. In women, VPA can also lead to androgenization, menstrual disturbances, and polycystic ovaries. The incidence of polycystic ovary syndrome (PCO) increases in women with epilepsy (WWE), which appears to vary with ethnicity.

Objective: Main aim of this study is determining a non-invasive biomarker in order early diagnosis of PCOs in WWE patients for preventative measures.

Materials and Methods: The study was carried out in 15 WWE women with PCO at reproductive ages (18-40 yr), monotherapy with VPA and 15 healthy women as control. Excluded criteria were history of diabetes, kidney and liver disease. After collecting patient' serum, total RNA was extracted and Q-RTPCR was used to identify the expressions level of miRNA-146.

Results: In accordance with previous studies on PCOs and non-PCOs women, expressions level of miRNA-146 were significantly different between epileptic patients with PCO and without it ($p<0.05$).

Conclusion: Our results suggesting miRNA-146 as non-invasive biomarker to screen for drug side effects and prevent the progression of its complications. Furthermore, bioinformatics analysis indicate role of miRNA-146 in insulin signaling pathway and increasing estradiol secretion which may be of importance in PCOs.

Key words: *Epilepsy, PCO, Non-invasive biomarker, miRNA-146.*

P-178

Smoking have a genetic predisposition and effect on infertility in women

Shirmohamadi M[1], Vahidi Mehrjardi MY[2].

1.*Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: maryam_shirmohamadi@yahoo.com

The high prevalence of smoking among women in their reproductive years continues to be a matter of concern. The negative effects of smoking on general health are familiar, but smoking may also affect fertility. About, 30% of women of reproductive age and 35% of men of reproductive age in the United States smoke cigarettes. In recent years, we have learned more about the role that genes play in the development of nicotine dependence. Studies show, that the strongest genetic contribution to smoking-related traits comes from variation in the nicotinic receptor subunit genes. Nicotinic receptor gene cluster on chromosome 15q25. Many other genes,

including those coding for enzymes involved in nicotine metabolism, also have been implicated. Therefore, it seems advisable to encourage the male to quit smoking before the attempt to conceive. Smoking is a familiar, risk factor of reproductive health. Available biologic, experimental, and epidemiological data indicate that up to 13% of infertility may be attributable to cigarette smoking. Smoking appears to accelerate the loss of reproductive function and may advance the time of menopause by 1 to 4 years. Currently, available evidence suggested a causal association between maternal smoking and reduced fertility. Some investigators reported that paternal smoking had a harmful effect on fecundity, but this was not confirmed by others. Interestingly, in a study of Chinese rural area shows husbands' smoking was associated with couples' infertility. We also observed a dose-response relationship that the associations increased with the increasing years of smoking, number of cigarettes smoked per day, and the total consumption of cigarettes.

Key words: *Smoking, Genetic predisposition, Infertility of women.*

P-179

Investigation the association of *SLC22A1* gene variants and efficiency of metformin on fetal development

Soltani S[1], Vahidi Mehrjardi MY[2], Kalantar SM[3].

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

Email: kalantarsm@ystp.ac.ir

Human placentas express several OCT isoforms (OCT1, OCT2, OCT3, MATE1 and MATE2). Among them Organic cationic transporter 1 (OCT1) that coded by gene SLC22A1 is one of the three similar polyspecific cationic transporters which principally expressed in the liver. Four nonsynonymous SLC22A1 variants that have most widespread been studied are rs12208357, rs34130495, OCT1 420 deletion (rs35167514, rs34305973, rs35191146, rs72552763), and rs34059508.

All four variants may have an effect on the pharmacokinetics and pharmacodynamics of metformin. metformin being the first therapeutic option for type 2 diabetes and it is also used to treat polycystic ovary syndrome in women. Either it may indirectly affect fetal development, through altered nutrient delivery or placental growth. In this review we investigate the association of SLC22A1 gene variants and efficiency of metformin on fetal development.

Key words: *SLC22A1 Polymorphisms, OCT, Fetal development, Metformin.*

P-180

Association of *BRCA1* mutations with serum anti-mullerian hormone levels: Connection between anti-mullerian hormone and ovarian cancer

Uosefvand B[1], Vahidi Mehrjardi MY[2].

Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: b301366@gmail.com

Ovarian cancer is the fifth most common cancer among women leading cause of cancer-related death among women. A woman's lifetime risk of dying from invasive ovarian cancer is 1 in 109 Survival rates tell you what percentage of people with the same type and stage of cancer are still alive (usually 5 yr) after they were diagnosed. More than one-fifth of ovarian tumors have hereditary susceptibility in about 65-85% of these cases. The genetic abnormality is a *germline* mutation in *BRCA* genes. *BRCA1* and *BRCA2* mutation carriers have an increased lifetime risk of developing ovarian cancer (up to 54% for ovarian cancer), as well as pancreatic and prostate cancer. On the other hand, recent studies have suggested that the *BRCA* mutation might be associated with occult primary ovarian insufficiency (POI). In some studies, on POI, the role of anti-mullerian hormone (AMH) has been demonstrated as a direct biomarker for ovarian aging and it is considered a quantitative marker of ovarian reserve. A case-control study has evaluated association of *BRCA1* mutations with AMH levels. This study showed that *BRCA1* carriers have lower serum AMH levels compared with women without *BRCA* mutations. Considering the important role of the BRCA genes in ovarian cancer as well as its significant association with serum AMH levels, several studies investigated the relationship AMH and ovarian cancer. Recently researches demonstrated an inhibitory effect of AMH on the proliferation, apoptosis and cell cycle of epithelial ovarian cancer cell lines. So that 10 μg/ml recombinant human AMH (rhAMH) was distributed to human OVCAR3 and OVCAR8 epithelial ovarian cancer (EOC) cell lines, then Cell proliferation, apoptosis, and cell cycle, as well subsequently level of stem cell factor (SCF) was investigated. This study concludes that rhAMH may be able to inhibit the proliferation and induce the apoptosis of EOC cells via G1/S phase cell cycle arrest and the decreased secretion of SCF. Future studies can focus on more examination the inhibitory effect of hormone on ovarian cancer cells.

Key words: *BRCA, Anti-mullerian hormone, Ovarian cancer.*

P-181

Association between G20210A prothrombin mutation and recurrent pregnancy loss in Iranian population of Yazd province

Zarein F[1], Hoseini SM[2], Montazeri F[1], Sheikhha MH[2], Rahmani S[2].

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

2.*Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: sheikhha@yahoo.com

Background: Recurrent pregnancy loss (RPL) has been inconsistently defined as 3 or more consecutive pregnancy losses prior to 20 wk from the last menstrual period. It affects approximately 1-2% of women. In the literature, there are many reports on the role of G20210A prothrombin mutation, as a thrombophilic disorder, in RPL.
Objective: The purpose of this study was to determine the association between G20210A mutation and RPL in 370 women with idiopathic RPL and 220 women without history of abortion and at least one term pregnancy as the control group.
Materials and Methods: Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen). Then, genotyping of G20210A mutation was carried out by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique.
Results: The G20210A mutation (heterozygote and mutant homozygote) was detected 10.9% in patients that is considerably higher in comparison with control group ($p<0.05$).
Conclusion: This study indicated the possible association of G20210A mutation with RPL. Therefore, it has been concluded that G20210A mutation screening would be useful to decrease the probability of RPL.
Key words: *Prothrombin, G20210A, ARMS-PCR, idiopathic RPL.*

P-182
Comprehensive study of chromosomal abnormalities in infertile patients referred to Cytogenetic laboratory of abortion research Center, Yazd Reproductive Science Institute; From March 2013 to January 2019

Zarezade Z, Karimi R, Golzade M, Dehghani MR, Kalantar SM.
Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
Email: smkyzd@gmail.com

Background: Numerical and Structural chromosomal abnormalities are recognized as a diagnostic factor in relation to infertility and recurrent pregnancy loss (RPL) and different responses to treatment. Infertility, RPL and being aware of the high risk for the genetic status of the fetus are one of the stressful complications in young couples with many psychological, physical and late complications. Cytogenetic analysis of chromosomal abnormalities could help choosing a better treatment for infertility through pre-diagnosis and post diagnosis.
Objective: According to relationship between infertility and chromosomal rearrangement, we aimed to survey frequency and different kinds of these abnormalities in patients referred to Cytogenetic Laboratory of Abortion Research Center, Yazd Reproductive Science Institute; From March 2013 to January 2019.
Materials and Methods: Medical records of 5,553 patients after genetic counseling with different indication include male and female infertility, RPL, IVF failure, pre-conception, pre-marital and ambiguous genital were studied. After performing G-banding karyotype in the cases, numerical and structural chromosomal abnormalities were analyzed.
Results: The results showed that 5064 patients (91.2%) had normal karyotype and 489 patients (8.8%) had abnormal karyotype. Karyotype report of patients revealed that pstk+ variation have the highest prevalence include 155 (31.9%) among studied karyotypes. Other abnormalities are as follows: Inversion: 70 patients (14.5%), Turner mosaicism 39 patients: (7.8%), Klinefelter: 34 patients (6.95%), Down syndrome:31 patients (6.35%), qh+: 30 patients (6%), Translocation: 23 patients (4.8%), qh-: 31 patients (6.35%) ,ps+ :14 patients (2.85%), Turner: 12 patients (2.45%), Robertsonian chromosome: 4 patients (0.8%), Sex reversal : 2 patients (0.4%), Klinefelter-Mosaicism: 11 patients (2.25%), Deletion: 10 patients (2%), Inversion/ Translocation: 1 patient (0.2%), Acrochromosome with cenh+: 3 patients (0.6%), Duplication/Inversion:1 patients (0.2%), Isochromosome: 1 patient (0. 2%), Trisomy 18: 2 patients (0.4%), Triple X: 3 patients (0.6%), Duplication: 10 patients (2%), Marker chromosome: 2 patients (0.4%).
Conclusion: The present study suggests that conventional karyotype testing can be used to identify the association between chromosomal abnormalities and infertility disorders and different responses to treatment, and the information obtained from tests for patient management, Genetic counseling and future plans for the patient are important.
Key words: *Infertility, RPL, Chromosomal abnormalities, Cytogenetic, Karyotype.*