

D- Poster Presentations

P-1

Effect of kindling with pentylenetetrazol on sexual hormone and sperm of male rats

Mehrabi Nasab E, Khazaei M.

Department of Fertility and Infertility, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.

E-mail: entezar148@yahoo.com

Introduction: Epilepsy is a disorder of cerebral neuron function. Clinical studies suggest a relation ship between epilepsy and Hypogonadism. The object of present work is to investigate recognition induced epilepsy effect by kindling with pentylenetetrazol (PTZ) on hormones and some reproductive parameters in male rat.

Materials and Methods: Adult male rat (NMRI strain) were divided in to control and kindling group. Kindling, was induced with 40 mg/kg PTZ with 48 hour interval by intraperitoneal injection. Each animal received 13 doses. At the end of thirteenth dose, animals were weighed and uthenaized with ether and blood were collected from heart for testosterone, prolactin and FSH, LH assay. In order to examination of motility and morphology of sperms, tissue samples from epididymis was isolated and is minced in DMEM/F12 culture medium and incubated for 15 minutes then sperm morphology and motility were studied . Testis were isolated and weighted. Data were analyzed with T-test method.

Results: Kindling with PTZ decrease serum level of testosterone, LH and increase prolactin. But there is no difference in serum level of FSH between two groups. sperm motility in kindling group significantly decrease. There is no significant difference in testis weight, but degree of animal weight losses of kindling group were seen.

Conclusion: Kindling with PTZ with change Serum level of sexual hormones and decrease sperm motility so it exerts diminishing negative effect on reproduction.

Key words: Kindling, PTZ, Rat, FSH, LH, Testosterone, Prolactin.

P-2

Role of heme Oxygenase-1 deficiency in recurrent IVF failure

Pirdel L¹, Pirdel M².

¹ Faculty of Medical Sciences, Islamic Azad University, Ardebil, Iran.

² Islamic Azad University, Astara, Iran.

E-mail: leilapirdel@yahoo.com

Introduction: For many couples experiencing infertility, IVF constitutes the last resort treatment, sometimes after other treatment options have also failed. Unfortunately, IVF is not always successful, and the cause for the implantation failure quite often remains unexplained. The cellular and molecular pathways leading to pregnancy maintenance have been intensively studied. From these studies, protective genes, as e.g., the Heme Oxygenase (HO) gene, were identified. It has been demonstrated that the activity of this enzyme is essential for modulating the inflammatory response, apoptosis, cellular proliferation and angiogenesis. Therefore, the HO-1 activity and expression as a diagnostic and therapeutic marker should be studied in patient with recurrent IVF failure.

Materials and Methods: Review of literature between 1998 to 2008.

Results: Heme oxygenase (HO)-1 may be involved in placental vascular proliferation and cell growth, since placental samples from patients suffering from spontaneous abortion, preeclampsia, choriocarcinoma or hydatiforme mole showed diminished HO-1 protein expression. It suggests that HO-1 might be essential for normal embryonic and placental development. Moreover, the down regulation of HO-1 expression at the feto-maternal interfaces in mice undergoing spontaneous abortion presented fibrosis and thrombosis areas in abortion samples. Down-regulated HO levels were related with increased Th1 cytokines production lead to fetal rejection. The up-regulation of HO-1 diminishes Th1-cytokines levels and inhibits TNF- α -induced apoptosis in fibroblasts. Interestingly, a regulatory interaction between inducible nitric oxide synthase (iNOS) to play an important role during pregnancy by producing nitric oxide and HO-1 has been described in several systems, since iNOS activation by cytokines seems to stimulate HO-1 expression.

Conclusion: In general, the underlying cause for IVF failure can be attributed to problems with the embryos, the uterine environment, or the patient's immune system. Then, HO-1 upregulation can be pregnancy- protective by deviating the Th1/Th2 ratio in Th2-favour or by protecting tissues from apoptosis in patients undergoing recurrent IVF failure.

Key words: Heme Oxygenase-1 Deficiency, Recurrent IVF Failure, Infertility.

P-3

Evaluation of endometrial biopsy histopathological results in Yazd infertile women

Mandegari M, Davar R, Akhavan Tafti AH.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medicine Sciences, Yazd, Iran.

E-mail: m_mndgr62@yahoo.com

Introduction: Infertility is generally defined as 1 year of unprotected intercourse without conception. Infertility therefore, affects approximately 10-15% of couples and women are responsible for 55 % of infertile patients. The endometrial biopsy is one of the complementary tests in recognizing the infertility cause that would determine some of the organic and functional disorders.

Materials and Methods: In this research 100 cases of infertile women were carried out. They were gone under endometrial biopsy in second half of the menstrual cycle for recognizing the cause of infertility. We also considered their treatment procedure and pregnancy outcome. Data were analysed by the SPSS software and the chi-square statistical test.

Results: The incidences of infertility causes that were recognized by the endometrial biopsy in this study were are the anovulatory phase (38%), luteal phase defect (11%) and chronic endometritis (17%). Overall in 66% of infertile women we were successful to diagnose the cause of infertility by endometrial biopsies. Regarding to the treatment by the clomifen-metformin, IUI, IVF and the medical treatment by the doxycyclin, there wasn't any considerable difference between the infertility causes that recognized by the endometrial biopsy. In this research there wasn't any considerable relation between the pregnancy outcomes with infertility cause and the infertility type, but the pregnancy outcomes has the considerable relation with age of the patients (p-value=0.038).

Conclusion: with regard to the nearly high percentage of luteal phase defect in this research (11%) and with regard to this point that we recognize the cause of infertility in more than half of the cases (66%) by endometrial biopsies, the endometrial biopsy should be considered as a useful screening test in infertile women but for determining it's position, it is better to compare endometrial biopsy with the other diagnostic tests such as the laparoscopy, sonography and the progesteron level of the blood.

Key words: Endometrial biopsies, Endometritis, Pregnancy.

P-4

Effects of different doses of Bone Morphogenetic Protein 4 on the viability and proliferation of CCE mouse embryonic stem cells

Makoolati Z¹, Movahedin M¹, Forouzandeh-Moghadam M².

1 Department of Anatomy, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.

2 Department of Biotechnology, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.

E-mail: zohreh1438@yahoo.com

Introduction: With regard to the importance of BMPs roles in the formation, development and function of various vital systems during fetal life, the aim of this study was to evaluate the effect of different doses of BMP4 on the viability and proliferation of CCE mouse embryonic stem (ES) cells.

Materials and Methods: CCE ES cells were trypsinized and cell suspension was prepared. The cells were counted and cultured in 96 well microplate. Each well of this plate containing 3×10^4 cells in 20% FCS in DMEM media. The cells incubated for 1 day, washed with PBS and cultured in DMEM containing different doses of BMP4 (1, 5, 25, 50 and 100ng/ml) as experimental groups. Control group was cultured in BMP4 free medium. ES cells incubated at 37°C overnight, washed with PBS, trypsinized and cell suspension was prepared separately from each well. In order to investigate the viability and proliferation rates of CCE ES cells, staining with trypan blue and counting were done. The mean number of whole cells and living cells were considered as proliferation and survival rates respectively. Data analysis was done with ANOVA test.

Results : No significant differences were found between the mean number of whole cells in the different doses (p=0.18), but the mean percent of living cells showed that BMP4 in 5 and 100 ng/ml concentration had the best and the worst effects on the viability of ES cells respectively (65.56 vs. 27.24%).

Conclusion: Evaluation of proliferation and viability rates using cell count and data analysis showed that addition of 5ng/ml BMP4 increased the proliferation and viability rates of CCE ES cells whereas high doses decreased these criteria. This suggests that different doses of BMP4

signaling may have different effects on ES cells behavior.

Key words: Embryonic stem cell, CCE, BMP4, Proliferation, Viability.

P-5

Improving damaged heart function in rabbit by transplantation of human umbilical cord matrix-derived mesenchymal stem cells with or without prior differentiation

Latifpour M, Nematollahi-mahani SN¹, Eftekhar-vaghefi Sh¹, Najafipour H², Deilami M³, Azimzadeh B³, Nabipour F⁴, Yaghoubi MM⁵, Eftekhar-vaghefi R⁶.

1 Department of Anatomy, Afzalipour School of Medicine, Kerman, Iran.

2 Department of Physiology, Afzalipour School of Medicine, Kerman, Iran.

3 Department of Cardiology, Shafa Hospital, Kerman, Iran.

4 Department of Pathology, Afzalipour School of Medicine, Kerman, Iran.

5 Hitech Center, Kerman, Iran.

6 Afzalipour School of Medicine, Kerman, Iran.

E-mail: payman936@yahoo.com

Introduction: Loss of cardiomyocytes after myocardial infarction is a causative factor in progression to heart failure because adult cardiomyocytes have essentially no regenerative capacity. Pathologic ventricular remodeling ensues as damaged myocardium is replaced by fibrous scar resulting in Ventricular function loss. In an effort to replace cardiomyocytes lost after infarction, cellular transplantation has been introduced as a potential therapy. The aim of present study was to investigate the potency of human Mesenchymal stem cells isolated from the human umbilical cord (hUCM).

Materials and Methods: Isolated hUCMs were treated with 5-azacytidine for cardiomyocyte differentiation. Thirty five adult Female New Zealand rabbits were divided into 5 groups: 1, intact; 2, control; 3, PBS; 4, undifferentiated human Umbilical Cord cells (hUCM) and 5, differentiated human Umbilical Cord cells (dhUCM). After anesthesia, rabbits were mechanically ventilated and, their hearts were exposed by means of a left thoracotomy through the fourth and fifth intercostals space. The proximal end of the left anterior descending coronary artery was ligated. After ligation for 1 h, 5×10^6 human Umbilical Cord Matrix cells in 50 μ l PBS, 5×10^6 cardiomyocyte-like cells in 50 μ l PBS

and 50 μ l PBS was carefully injected at the border area of the ischemic myocardium respectively in hUCM, dhUCM and PBS groups. Echocardiography, general pathology and immunohistochemistry were performed in order to detect any myocardial regeneration and improvement of cardiac function.

Results: 5-azacytidine Treated cells in comparison to non-treated group were positive for F-actin and negative for troponin I. The rate of ejection fraction, diameter of left ventricular wall at the end of diastole and systole was significantly different in cell transplanted groups when compared to PBS (p=0.001) and control (p=0.002) groups. A significant reduction in the fibrous tissue and fibroblasts occurred in cell-transplanted groups versus PBS and control groups. Surviving hUCM and dhUCM were identified by BrdU positive spots in infarcted region and few transdifferentiated cells into cardiomyocytes were characterized with a positive cardiac phenotype: troponin I and human F actin.

Conclusion: Our data show that mesenchymal stem cells from Wharton's jelly may improve heart function and undergo differentiation to cardiomyocyte-like cells following transplantation. These cells may provide an appropriate source of cells for cell therapy procedures.

Key words: Human umbilical cord matrix cells, Cardiomyocytes, 5-azacytidine, Heart function.

P-6

Effect of Doxepin on physiology of reproduction in adult male Rat

Shariati M, Mokhtari M, Amiri F.

Islamic Azad University, Kazeroun, Iran.

E-mail: mehrdadshariati@hotmail.com

Introduction: Doxpin is a serotonin and norepinephrine reuptake inhibitor. Considering the importance of this drug in treating nervous diseases, its side effects are very important on the endocrine axis. In this research the effect of Doxepin were studied on the concentration of testosterone, FSH and LH level and spermatogenesis.

Materials and Methods: The experiments were done on 40 male Wistar rats that divided to 5 groups of 8. The control group received nothing. The sham group was given distilled water as a solvent. The experimental groups were injected 35, 70 and 140 mg/kg of the drug orally for 21 days. The blood samples were taken at 22^d day and the

concentration of testosterone, FSH and LH were measured by RIA method. In addition, at the 22^d day, the testes were separated and histological changes were studied among experimental, sham and control group. The results were evaluated by using ANOVA and Duncan tests.

Results: The results showed that 140 mg/kg of Doxepin reduced serum testosterone level while it increased FSH and LH levels ($p < 0.05$). Histological investigations of the testes showed a decline on spermatogenesis chain in dose of 140 mg/kg.

Conclusion: According to our findings, Doxepin decreases the concentration of testosterone level and the number of spermatogenic cells and increases FSH and LH levels at high doses. Also, it can weaken the function of reproductive activity, probably.

Key words: Doxepin, Reproduction, Rat.

P-7

In vitro application of hepatocyte growth factor promote mouse blastocyst formation

Ghasemian F¹, Bahadori MH², Azarnia M³, Nasiri E², Ghasemi F².

Faculty of Science, Tarbiat Moallem University, Tehran, Iran.

2 Guilan University of Medical Sciences, Faculty of Medicine, Dept. of Anatomy, Rasht, Iran.

3 Tarbiat Moallem University, Tehran, Iran.

E-mail: yasaman_21_a@yahoo.com

Introduction: Research studies on reproductive mechanism of laboratory animals are essential for further advancement of assisted reproductive techniques (ART). One of these studies includes the assessment of in-vitro development of pre-implantation embryos. The objective of this study was to compare the cleavage rates and morphology of formed Two pronucleous (2 PN) zygotes to blastocyst and hatching blastocyst stage in drops of T6 medium with or without HGF (10, 20, 50 and 100 ng/ml).

Materials and Methods: 6-8 weeks old female NMRI mice were superovulated with 5IU pregnant mare's serum gonadotropin (PMSG, ip) and subsequent human chorionic gonadotropin (hCG, ip). Superovulated animal were caged with male mouse for mating. Mated mice were killed by cervical dislocation to collect a total of 2 PN zygotes from oviduct and were cultured to the hatched blastocyst stage and the number of embryo in different stage was recorded under an invert microscope and compared.

Results: In this study, Addition of 20 ng/ml HGF to the culture medium significantly ($p < 0.05$) increased the percentage of 2 PN mouse embryos that developed into blastocysts, but culture of embryos in drops of T6 medium with HGF (20 ng/ml) had no effect on hatching blastocyst development. Also, in the presence of 100 ng/ml HGF, the development rate was significantly decreased.

Conclusion: Exogenous HGF at low concentration promote mouse blastocyst formation in vitro.

Key words: Hepatocyte growth factor, In vitro preimplantation development, Cleavage, Pronucleous, blastocyst.

P-8

Back muscle versus kidney capsule for ovarian transplantation, an animal model

Oskouian H, Ahmadi Sh, Khalili MA, Miresmaeili SM.

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

E-mail: HomaOskouian@gmail.com

Introduction: The aim of this study was to evaluate the optimal transplantation site for ovarian tissue fragments in murine hosts. We compared the transplantation to the back muscle (B) versus the kidney capsule (K) in a mouse allograft model.

Materials and Methods: Hemi ovaries from 12-day-old mice were allografted into B and K same strain recipients which had undergone gonadotropin stimulation. Graft survival after 21 days and follicle development were scored and compared to age-matched control ovaries.

Results: Premordial follicular and survival was higher in K – than in B – grafts.

Conclusion: The kidney capsule is better than back muscle for ovarian allografts in mice.

Key words: Back muscle, kidney capsule, Ovarian transplantation, Animal model.

P-9

Effect of milk and glycerol extender on vitality and motility of Najdi bull sperm in 5 degrees of centigrade in different time duration of exposure

Afrough M^{1,2}, Barati F¹, Papahn A¹.

1 Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.

2 Allam-Al-Hoda Research Center, Baseej Deneshjoie – Khuzistan, Iran.

E-mail: m.afrough@gmail.com

Introduction: Storage of semen in refrigerator is one of possible method to preserve it. This experiment was designed to evaluate the effect of addition of glycerole in milk extender and duration of exposure in 5°C on bull sperm motility and viability.

Materials and Methods: Semen collected from 5 Najdi Bulls in station for support of Najdi cow, Khuzistan, Iran. The experiment was conducted at 5°C to test sperm reaction at the typical prefreeze processing temperature, using Milk extender with and without glycerole (using pasteurised milk from Choopan Industry). Semen was centrifuged and after remove of supernatant, centrifuged semen was mixed by equal volume of the extender. Motility and viability were analysed after 15 and 60 minute of exposure.

Results: The percentage of vitality was decreased in both M+ and M- extenders and there was no significant difference between them. Motility rate was decreased and was not affected with duration incubation in milk extender but glycerole supported viability in 15 minutes after exposure.

Conclusion: After 60 min. of incubation milk extender had no effect but addition of glycerole had negative effect on vitality.

Key words: Milk, Glycerol, Vitality, Najdi bull sperm.

P-10

The effects of crowding stress on mouse uterus horn and fallopian tube epithelium

Sabbagh Ziarani F, Rajaei F, Javadi A.

Department of Anatomy, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.

E-mail: fateme_2008z@yahoo.com

Introduction: Increase in the number of animals per cage is a very important stress factor. The stress response begins as changes in tissue and organ function that resulting in a move away from homeostasis. Since female experience different types of stress than male due to their biological differences that often associated with changes in reproductive system function, in the present study the effects of crowding stress on height of epithelial cells in mouse endometrium and fallopian tube were investigated.

Materials and Methods: 25 female mice were randomly divided into 2 groups. Control group (3 cages) with 5 mice per cage and experimental group (1 cage) with 10 mice per cage were kept for 1 month. Female mice in both groups were superovulated and killed at the time of ovulation

(12 h post hCG). The samples of Uterus horns and fallopian tubes in all groups were taken and were processed for light microscopic studies. The data has been compared using statistical methods (SPSS, Mann-Whitney test and $p < 0.05$).

Results: The analysis of the height of fallopian tube epithelial cells in term of micrometer in experimental groups showed significant increase in compare to control group (36.4 ± 4.4 , 27.3 ± 3.1 $p < 0.001$). However there was no significant difference in the height of endometrial epithelial cells between two groups (23.8 ± 2.03 , 23.3 ± 3.4 $p = 0.39$).

Conclusion: The results indicated that the crowding stress may affect the female reproductive system in mice by increasing the height of fallopian tube epithelial cells.

Key words: Crowding stress, Epithelial cell, Endometrium.

P-11

The effect of mobile wave on pregnant mouse

Mriresmaeili SM, Fesahat F, Sadeghian F.

Research and Clinical Center for infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

E-mail: Smm230@gmail.com

Introduction: One of the most practical tools in the world is mobile phone. In recent studies reported that the imagery mobile wave have harmful effects on human and animals .However, these researches used imagery mobile wave, but the effects of real mobile on humans or animals have not been evaluated until now. Therefore, in this study the mobile phone has been used as a wave producer.

Materials and Methods: This study was done on 20 pregnant female mice, divided in 2 groups (10 control and 10 experimental). The experimental group (10 mice) has been exposed to mobile phone waves in matting time until breeding. The mobile phones were turn on during the days and have been used at least an hour every day.

Results: In this study we didn't see any difference between experimental and control group in outward character, breeding time, litter weight, litter number and litter genus.

Conclusion: The imagery mobile waves have harmful effects on human and animal body but in this study we did not see any abnormality in mice pups.

Key words: Pregnancy, Mouse, Mobile phone.

P-12

Interspecies embryo transfer in camelids: the birth of the first Bactrian camel calves (*Camelus bactrianus*) from Dromedary camels (*Camelus dromedarius*)

Niasari-Naslaji A¹, Nikjou D¹, Skidmore JA², Moghiseh A¹, Mostafaey M³, Razavi K⁴, Moosavi-Movahedi AA⁵.

1 Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran.

2 The Camel Reproduction Centre, Dubai, UAE.

3 Research Centre for Agriculture and Natural Resources, Ardabil, Iran.

4 Animal Science Research Institute, Karaj, Iran.

5 Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.

E-mail: niasari@ut.ac.ir

Introduction: Interspecies embryo transfer is a possible approach that can be used to conserve endangered species. It could provide a useful technique to preserve the Iranian and wild Bactrian camels, both of which are threatened with extinction.

Materials and Methods: In the present study, one Bactrian camel was superovulated using decreasing doses of FSH (60, 40, 30, 30, 20, 20 mg, b.i.d.; Folltropin-V; Bioniche, London, ON, Canada) for 6 days, followed by a single injection of FSH (20 mg, i.m.) on Day 7. Daily ovarian ultrasonography was performed until most of the growing follicles had reached a mature size of 13–17 mm, at which time the camel was mated twice, 24 h apart, with a fertile male Bactrian camel. At the time of first mating, female camels were given 20 µg, i.v., busserelin (Receptal; Intervet, Boxmeer, The Netherlands). One day after the donor camel had been mated, the dromedary recipients (n=8) were injected with 25 mg, i.v., porcine LH (Lutropin-V; Bioniche) to induce ovulation. Embryos were recovered on Day 8.5 after the first mating and transferred non-surgically into recipients on Day 7.5 after LH injection.

Results: Pregnancy was diagnosed 25 days after embryo transfer. Healthy Bactrian camel calves (n=4) were born without any particular complications at the time of parturition (e.g. dystocia and neonatal diseases).

Conclusion: The present study is the first report of the birth of Bactrian camel calves from dromedary camels, as well as the first report of interspecies embryo transfer in old world camelids.

Key words: Interspecies embryo transfer, Camelids, *Camelus bactrianus*, *Camelus dromedarius*.

P-13

Neurogenic and mitotic effects of dehydroepiandrosterone on neuronal-competent marrow mesenchymal stem cells

Hashemi Shiri E^{1,2}, Zare Mehrjardi N¹, Tavallaei M², Kazemi Ashtiani S¹, Baharvand H^{1,3}.

1 Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran.

2 Department of Biology, Imam Hossein University, Tehran, Iran.

3 Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

4 Department of Stem Cells, Cell Science Research Center, Royan Institute, Tehran, Iran.

E-mail: Baharvand50@yahoo.com

Introduction: To establish whether Dehydroepiandrosterone (DHEA) as a neurosteroid could enhance the rate of neuronal differentiation in neuronal-competent bone marrow mesenchymal stem cells (BM-MSCs).

Materials and Methods: We have added DHEA at two stages of before and after plating the neurosphere-like aggregates.

Results: Flow cytometric analysis of Tubulin-III and Tau positive cells revealed that the percentages of these cells were increased significantly for the two markers following DHEA treatment in both stages.

Moreover, Western blot analysis has revealed that Tubulin-III protein was strongly induced by DHEA. The expression of neuronal specific genes such as Isl-1, Tubulin III, Pax6, and Nestin was also detected by RT-PCR analysis as well as BrdU incorporation and found to have increased significantly after DHEA induction.

Conclusion: These results have presented evidence that DHEA can affect neuronal-competent MSCs in inducing the expression of a comprehensive set of genes and proteins that define neuronal cells. DHEA was also able to induce the division of neuronal-competent MSCs and therefore increasing the number of cells with major neuronal characteristics.

To our knowledge, this is the first report that DHEA has been shown to induce the division and differentiation of MSCs into neurons in vitro and should provide a better insight into the treatment of a wide variety of neurological diseases by MSCs.

Key words: Dehydroepiandrosterone, Mesenchymal stem cells, Neuronal differentiation.

P-14

Relationship of sperm morphological abnormalities with levels of Reactive Oxygen Species in semen specimens

Nasr Esfahani M, Moein MR, Talebi AR, Ghasemzadeh J.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medicine Sciences, Yazd, Iran.

E-mail: dr.matin.nasr@gmail.com

Introduction: Reactive Oxygen Species (ROS) are a group of free radicals in human semen that its excessive formation has been associated with impaired sperm morphology and fertility potential. We compared ROS levels in seminal plasma of infertile men with this level in healthy donors. We also determined the ROS level association with percentages of different sperm morphological abnormalities, the sperm deformity index, and the teratozoospermic index scores.

Materials and Methods: In total 30 infertile patients and 25 healthy donors as control were selected. Sperm analysis was done for all patients. Azoospermic patients were excluded from the study. ROS level in semen were measured by a chemiluminescence assay in both group. Thin smears of well-mixed semen were stained with papanicolaou to assess morphological abnormalities.

Results: The mean ROS level in normal men was 166.10 RLU (Relative Light Unit) while this was 1630.50 RLU in infertile patient which is significantly higher in case group ($p=0.000$). A significant positive correlation was observed between sperm ROS production and the proportion of sperm with abnormal morphology. ROS production was positively correlated with the proportion of sperm with amorphous head, midpiece defects, cytoplasmic droplets, tail defect, pinhead sperms, SDI scores, and TZI scores ($p<0.005$).

Conclusion: The level of ROS in seminal fluid of infertile men is significantly higher than fertile donors and also sperm morphological abnormalities, SDI score and TZI score are useful to predict levels of ROS.

Key words: Male infertility, Reactive Oxygen Species (ROS), Sperm morphology.

P-15

The effects of electromagnetic field on DNA fragmentation in mouse blastocyst

Borhani N, Rajaei F, Sabbagh Ziarani F, Javadi A.

Department of Anatomy, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.

E-mail: nsm_borhani@yahoo.com

Introduction: Life on earth has evolved in a sea of natural electromagnetic fields (EMFs). Over the past century, this natural environment has sharply changed with introduction of a vast and growing spectrum of man-made EM fields. Human data reviewed concern the potential reproductive effects of exposure to various sources of EMFs.

Materials and Methods: 80 female mice were randomly divided into 2 groups; control group was not exposed to EMF and EMF group was exposed to 4 hours per day, 6 days a week for 2 weeks to 50 Hz & 0.5 mT EMF. Female mice in Control group and exposed group on 8th day of exposing were superovulated with PMSG and hCG and mated over the night. Next morning females with a vaginal plug were identified as pregnant mice; 102 h after hCG injection pregnant mice were anaesthetized with cervical dislocation. Blastocysts were subsequently obtained from these mice by flushing the uterus horns. After fixation, Blastocysts were stained with TUNEL technique. Then stained Blastocysts were analyzed with florescent microscope from number of blastomers and apoptotic cells. Pregnancy rate, number of fetuses per litter, number of blastomers and DNA fragmentation index in control & EMF groups were analyzed statistically.

Results: Results showed that the mean number of pregnant mice decreased in EMF group compared to the control group but the difference between them was not significant (67.5%, 50% and $p=0.086$). The mean number of fetuses per litter was 9 ± 4.8 in control group and 5.5 ± 5.7 in EMF group and statistical analysis were showed significant decrease between mean of 2 groups ($p<0.02$). Numbers of blastomers in each Blastocyst were decreased in EMF group compared to the control group but this decrease was not significant (45.94 ± 7.3 , 48.3 ± 7.2 and $p=0.06$). DNA fragmentation index was 7.14% in control and 10.53% in EMF group that this decrease was significant ($p<0.001$).

Conclusion: These results showed that EMF has detrimental effects on the female reproduction and embryo development.

Key words: Electromagnetic field, Mouse blastocyst, DNA fragmentation.

P-16

Studying teratogenic and abortifacient effects of different doses of saffron (*Crocus Sativus*) decoction in 1st or 2nd trimesters in mice

Hosseini SM¹, Dashti MH², Anvari M³, Zeinali F⁴, Miresmaeili SM⁵.

1 Faculty of Pharmacy and Pharmaceutical Sciences, Esfahan University of Medical Sciences, Esfahan, Iran.

2 Department of Physiology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

3 Department of Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4 Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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

E-mail: smhbyazd@gmail.com

Introduction: Beside several economical benefits Saffron has been considered as a medicinal plant since ancient times. However there are some evidences about it's abortifacient effects especially in high doses. The aim of present study was to assess the effects of non toxic doses of aqueous saffron decoction (0.8, 0.2, 0.4 %) on abortion and congenital disorders in mice.

Materials and Methods: In this study 35 female BALB/c mice, weighting 25-30 grams, were randomly divided into 7 equal groups. Breeding of animals were done in the animal house of medical college by keeping the ratio of male: females as 1:5, in each plastic cage. The females were checked daily for vaginal plaque and the day on which the vaginal plaque was observed, was considered as the first day of pregnancy. The pregnant rats were divided into 7 subgroups and placed in separate cages throughout the gestational period and were acclimatized and fed in the same conditions. Animals in control group received tap water but the test groups received different concentrations (0.8, 0.2, 0.4) of aqueous saffron extract in 1st or 2nd trimesters throughout the gestational period. In 20th day of pregnancy, animals were anesthetized and their fetuses were extracted through a cesarean section. The placenta was excised, weighed, and the number and placement of implantation sites, Live and dead fetuses and early and late resorptions were recorded. All fetuses were stereo microscopically examined for any morphological abnormalities.

Results: According to our findings placental weight and diameter, mean fetal weight, body and tail length and biparietal diameter in all test groups were smaller than those in control group and these

differences were significant in animals receiving 0.8% saffron solution and mostly those who were received the decoction on 2nd trimester. The mean numbers of resorbed and dead fetuses in test groups were dose dependently greater than control group. Maximum resorbed fetuses were observed in animals receiving 0.8% saffron solution on 1st trimester while the maximum dead fetuses were for animals receiving 0.8% saffron solution on 2nd trimester.

Conclusion: since embryonic implantation occurs in the first gestational trimester, saffron and its components may affect this phenomenon and may result in abortion.

Organogenesis is mostly happens in the 2nd gestational trimester and the effect of saffron specially in high doses may interfere this event and lead to abnormalities such as decrease in tail length and in placenta weight and diameter

According to our findings saffron, is not a safe herb for using in gestational period specially in high doses.

Key words: Saffron (*Crocus Sativus*), Abortion, Morphological abnormalities.

P-17

The effects of LIF on mouse preimplantation embryo development

Parvini M², Amiri I¹, Amini A².

1 Hamadan University of Medical Sciences, Hamadan, Iran

2 Department of Biology, Faculty of sciences, Razi University, Kermanshah, Iran.

E-mail: mpparvini@yahoo.com

Introduction: Recent studies have demonstrated that mammalian preimplantation embryos are exposed to a mixture of many different growth factors and cytokines, expressed by the follicles, oviducts and endometrium. Receptors for many of these growth factors have also been shown to be expressed by preimplantation embryos. In vitro culture of human and animal's embryos in conventional media lacking growth factors can result in suboptimal growth and a variety of short-term and long-term developmental abnormalities. One of these factors is Leukemia inhibitory factor (LIF). The aim of this study was to evaluate the effects of LIF on the mouse preimplantation embryo development.

Materials and Methods: Six to eight weeks old NMRI mice were superovulated by injection of 10IU PMSG and 10IU HCG 48h later. The mated mice were killed 48 hours after hCG injection,

oviducts were flushed and two-cell embryos collected and divided randomly to two groups (Control and treatment). Control medium was HTF and treatment medium was HTF+1000u/ml LIF. In each group the embryos were cultured in an incubator at 37°C with 5% CO₂ for 72h. The state of embryo development was evaluated in 12 hours interval using inverted microscope.

Results: There was not any significant difference in the rate of morolla and blastocyst formation after 36 hours. In comparing hatching rates, 60 and 72 hours after culture, there were significant difference between control and treatment groups ($p < 0.008$).

Conclusion: LIF doesn't provide obvious stimulation in the early mouse embryo development until morolla stage; however, it has positive effects on preimplantation blastocyst growth, differentiation and hatching.

Key words: LIF, Preimplantation embryo, Mouse.

P-18

Administration of ecstasy during the pregnancy on mouse ovary

Lotfi Hormozdabadi M, Khalili MA, Akhavan M, Mortazavi MH, Mola Abasi AR.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

E-mail: zahed_masih@yahoo.com

Introduction: One of the most important periods of a woman's life is pregnancy. Drug abuse can have effects on this period. The effect of ecstasy on fertility is unknown. We decided to examine effects of administration of ecstasy during the pregnancy on mouse ovary.

Materials and Methods: This study is a experimental research. We had 15 female mouse in case group and 5 female mouse in control group that became pregnant. We inject 5mg/kg of ecstasy to case group and normal saline to control group (consideration of stress factor) in 7th and 14th day of pregnancy. 27 days after delivery we eliminate mice and cut ovaries out. Ovaries send to pathology lab for sectioning and staining, after that we study microscopic and macroscopic changes that happen to ovaries.

Results: In sections that provided by lab, primary follicles in case group was less than control group (p -value=0.014). Mature follicles in case group was more than control group (p -value= 0.039). Luteal follicles in case group was less than control

group (p -value= 0.016). Other follicles do not show valuable results.

Conclusion: Base on this results there is an unknown mechanism (may be CNS hormones) that exacerbate maturation of primary follicles and reduce lutealization.

Key words: Ecstasy, Pregnancy, Ovary, Mouse.

P-19

Effect of Leukemia Inhibitory Factor and Epidermal Growth Factor on mouse oocyte maturation

Mirahadi N¹, Heidarbeigi Kh¹, Parvini M¹, Amiri I², Amini A¹.

1 Department of Biology, Faculty of Sciences, Kermanshah University, Kermanshah, Iran.

2 Department of Anatomy, Medical School, Hamedan University of Medical Sciences, Hamedan, Iran.

E-mail: nsm_1360@yahoo.com

Introduction: As we know assisted reproductive technology has become the frontier of infertility treatment. Also, the core of an assisted reproduction program is oocyte quality because one of the best ways to improve embryo quality is to improve oocyte quality. There are several ways to improve the quality of maturation and development of embryos, one way is using of different factors in media such as; cytokines and growth factors. This study was, therefore, set up to investigate the effect of LIF together with EGF on rate of maturation and fertilization of mouse oocytes.

Materials and Methods: Germinal vesicle oocytes were randomly assigned to four treatment groups. The base culture medium was Waymouth. Oocytes in treatment groups were cultured in the same medium supplemented with 1000IU/ml leukemia inhibitory factor (Treatment 1), 10ng/ml recombinant human epidermal growth factor (Treatment 2), and 1000IU/ml LIF+ 10ng/ml rhEGF (Treatment 3). After 24 and 48 h on treatment, the significance of differences in maturation was evaluated by the one way analysis of variance. Step 2: MII oocytes were randomly divided to four treatment groups. The base culture medium was HTF. Oocytes in treatment groups were cultured in the same medium supplemented with 1000IU/ml LIF (Treatment 1), 10ng/ml rhEGF (Treatment 2) and 1000IU/ml LIF+ 10ng/ml rhEGF (Treatment 3). After that capacitated spermatozoa were added to every drop.

After 4-6 h all of the oocytes transferred in HTF+ 10% HSA for culture.

After 24 and 48 h embryonic development to 8-cell were evaluated and data were evaluated using one way analysis of variance.

Results: In the EGF group, maturation improved significantly than LIF group ($p < 0.05$). On the other hand the rate of 8-16 cell embryos formation after 48 h culture in LIF group was significantly higher than those supplemented with EGF.

Conclusion: Therefore, in vitro maturation of GV oocytes was improved in the presence of EGF but LIF is able to improvement of fertilization.

Key words: LIF, EGF, IVM, Embryo, Mouse.

P-20

Effect of Tamoxifen on differentiation and survival of oocytes and folliculogenesis in the mouse

Afsordeh K¹, Roshangar L², Soleimani Rad J³.

1 Department of Histology, Tabriz University of Medical Sciences, Tabriz, Iran.

2 Neuroscience Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran.

3 Tissue Engineering Team, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: kobra.afsordeh@yahoo.com

Introduction: During gonadal development, after migration of primordial germ cells to gonadal site, they divide and differentiate to oogonia. Oogonia divide repeatedly and form oocytes which will later on develop to primordial follicles. Prior to follicular development, they have to go through oocyte nest formation and breakdown. Any disturbances in this process would result in poor development of ovarian reservoir and folliculogenesis disorder in adulthood. The aim of the present study is to investigate the effect of Tamoxifen, a estrogen receptor binding agent, on oocyte nest formation and breakdown.

Materials and Methods: In this study 30 adult female and 15 adult male mice are used. The female mice are divided into two groups of control and experimental. At their sterous cycle, two female mice were housed in a cage with one male mouse for mating. Formation of vaginal plaque was considered as the first day of pregnancy. Since the thirteenth day of pregnancy each mouse received 100g Tamoxifen as ip injection. After delivery, the 2, 3, 6 and 7 days old new borns were sacrificed and their ovaries were fixed and prepared for light microscopic studies. In the

sections the formation and diameter of oocyte nests and the number of primordial and primary follicles were determined and compared in controls with experimental.

Results: Oocyte nests were recognized on second and third day new borns and their diameter in experimental group were significantly higher than in control group. On the sixth and seventh days old infants, the numbers of primary multilayered follicles were significantly lower than those in control group.

Key words: Tamoxifen, Oocytes, Folliculogenesis, Mouse.

P-21

Studing teratogenic and abortificant effects of different doses of saffron (Crocus Sativus) decoction in whole gestational period and the 3rd trimester of gestational period in mice

Zeinali F¹, Dashti MH², Anvari M³, Hosseini SM⁴, Miresmaeili SM⁵.

1 Medical School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2 Department of Physiology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

3 Department of Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4 Faculty of Pharmacy and Pharmaceutical Sciences Isfahan University of Medical Sciences, Esfahan, Iran.

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

E-mail: smhbyazd@gmail.com

Introduction: Beside several economical benefits, Saffron has been considered as a medicinal plant since ancient times. However there are some evidences about it's abortificant effects especially in high doses. The aim of present study was to assess the effects of non toxic doses of aqueous saffron decoction (0.8, 0.2, 0.4 %) on abortion and congenital disorders in mice.

Materials and Methods: In this study 35 female BALB/c mice, weighting 25-30 grams, were randomly divided into 7 equal groups. Breeding of animals were done in the animal house of medical college by keeping the ratio of male: females as 1:5, in each plastic cage. The females were checked daily for vaginal plaque and the day on which the vaginal plaque was observed, was considered as the first day of pregnancy. The pregnant rats were divided into 7 subgroups and placed in separate cages throughout the gestational period and were acclimatized and fed in the same conditions. Animals in control group received tape water but the test groups received different

concentrations (0.8, 0.2, 0.4) of aqueous saffron extract in whole gestational period and 3rd trimester throughout the gestational period. In 20th day of pregnancy, animals were anesthetized and their fetuses were extracted through a cesarean section. The placenta was excised, weighed, and the number and placement of implantation sites, Live and dead fetuses and early and late resorptions were recorded. All fetuses were stereo microscopically examined for any morphological abnormalities.

Results: According to our findings placental weight and diameter, mean fetal weight, body and tail length and biparietal diameter in all test groups were smaller than those in control group and these differences were significant in animals receiving 0.8% saffron solution and mostly those who were received the decoction in whole gestational period. The mean numbers of resorbed and dead fetuses in test groups were dose dependently greater than control group. Maximum resorbed and dead fetuses were observed in animals receiving 0.8% saffron solution in whole gestational period rather than those received the solution on 3rd trimester.

Conclusion: since embryonic implantation occurs in the first gestational trimester, saffron and its components may affect this phenomenon and may result in abortion.

Organogenesis is mostly happens in the 2nd gestational trimester and the effect of saffron specially in high doses may interfere this event and lead to abnormalities such as decrease in tail length and in placenta weight and diameter. All these abnormalities were seen on using the solutions in whole gestational period. No severe special morphological abnormalities were seen in animals received the saffron extracts on 3rd trimester. According to our findings saffron, is not a safe herb for using in gestational period especially in high doses.

Key words: Saffron (*Crocus Sativus*), Abortion, Morphological abnormalities.

P-22

Dietary fatty acid intakes and the risk of ovulatory infertility

Akhbardeh M.

Boston medical group, Boston, USA.

E-mail: mahdi_akhbardeh14@yahoo.com

Introduction: Pharmacologic activation of the peroxisome proliferator-activated receptor α (PPAR- α) improves ovulatory function in women with polycystic ovary syndrome, and specific dietary fatty acids can affect PPAR- α activity. The

objective of the study was to assess whether the intakes of total fat, cholesterol, and major types of fatty acids affect the risk of ovulatory infertility.

Materials and Methods: We conducted a prospective cohort study of 18555 married, premenopausal women without a history of infertility who attempted a pregnancy or became pregnant between 1991 and 1999. Diet was assessed twice during follow-up by using a food-frequency questionnaire.

Results: During follow-up, 438 incidents of ovulatory infertility were reported. In logistic regression analyses, intakes of total fat, cholesterol, and most types of fatty acids were not related to ovulatory infertility. Each 2% increase in the intake of energy from *trans* unsaturated fats, as opposed to that from carbohydrates, was associated with a 73% greater risk of ovulatory infertility after adjustment for known and suspected risk factors for this condition [relative risk (RR)=1.73; 95% CI: 1.09, 2.73]. Obtaining 2% of energy intake from *trans* fats rather than from n-6 polyunsaturated fats was associated with a similar increase in the risk of ovulatory infertility (RR=1.79; 95% CI: 1.11, 2.89). In addition, obtaining 2% of energy from *trans* fats rather than from monounsaturated fats was associated with a more than doubled risk of ovulatory infertility (RR=2.31; 95% CI: 1.09, 4.87).

Conclusion: *trans* Unsaturated fats may increase the risk of ovulatory infertility when consumed instead of carbohydrates or unsaturated fats commonly found in nonhydrogenated vegetable oils.

Key words: Diet, Dietary fatty acids, Infertility, Ovulation, Reproductive medicine, Nutritional epidemiology.

P-23

Stimulation of osteogenesis in “Mesenchymal Stem Cells” by combined bone morphogenetic Protein – 2, Dexametasone and Dihydroxy-Vitamin D

Owringi B, Reilly G.

Biomaterial Engineering, Kroto Institute, The University of Sheffield, UK.

E-mail: Bahar7667@gmail.com

Introduction: In vitro experiments with Mesenchymal Stem Cells (MSC) are beneficial for better understanding of bone biology and bone formation. Our aim is to examine and stimulate hMSC differentiation into osteoblasts by different inducers either in serum or serum free medium.

Materials and Methods: In this investigation, we carried out experiments on human MSC obtained from bone marrow of a healthy 44 year old male. hMSC were treated with either 10⁻⁸M 1, 25-dihydroxyvitamin D (vitamin D), 10⁻⁷M Dexametasone, 100 ng/ml BMP-2 or in combinations in 2D cultures and as a new work in 3D polyurethane scaffolds. The osteoblast induction was determined by alkaline phosphatase (Alp) activity, calcium and collagen production, osteopontin, collagen type 1 and Msx2 expressions.

Results: BMP-2, Dex and Vit.D treatment increased Alp activity, Msx2 and osteopontin expressions but BMP-2 alone had less effect on Alp activity in 3D cultures in serum medium however Alp activity was increased with BMP-2 alone in serum free conditions in 2D and 3D cultures. According to our results Alp Activity was highest with combination of all three treatments either in serum or serum withdrawal in both 3D and 2D cultures of hMSC. BMP-2 alone or in combination with Dex and Vit.D enhanced calcium and collagen production in both serum and serum free medium. In the end, *in vitro* mineralization was significantly increased in hMSC cultures treated with these inducers. Cell proliferation was greater increased by BMP-2 than Dex treatment either in serum free or serum medium.

Conclusion: To recapture briefly, hMSC can be differentiated into osteoblast with Dex, BMP-2 and Vit.D inducers *in vitro*. However, these three stimulators produce different characteristics osteoblastic phenotypes with different levels of early genes expressions in serum and serum free medium in either 2D or 3D hMSC cultures which indicates the interactions with other signaling pathways during this induction.

Key words: Osteogenesis, Mesenchymal Stem Cells, Dexametasone, Dihydroxy-Vitamin D.

P-24

Significance of human chorionic gonadotropin (hCG) in the growth and maturation of preantral follicles and enclosed oocytes during optimized *in vitro* cultures: a randomized study

Javed A^{1,4}, Rezaei-Zarchi S², Anvari M¹, Javeed Ghani M³, Jamil A⁴, Kalantar SM^{1,5}.

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

² Department of Biology, Payam-e-Noor University, Yazd, Iran.

³ Department of Bioinformatics, Government College University, Faisalabad, Pakistan.

⁴ Molecular Biochemistry Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

⁵ Yazd Medical Biotechnology and Genetic Engineering Incubator, Yazd, Iran.

E-mail: smkalantar@yahoo.com

Introduction: Fertilization treatment using the oocytes, matured *in vitro*, has many potential applications. It minimizes the risk of severe ovarian hyper-stimulation. The study was carried out to evaluate the effect of hCG, in the presence and absence of FSH, on the timing and regulation of *in vitro* ovulation in Syrian mice preantral follicles.

Materials and Methods: Preantral follicles, isolated from the ovaries of 6 week-old mice, were cultured in TCM-199 medium. The effect of 10-200 mIU/ml FSH and 1.5 IU/ml hCG, the follicles were incubated for 6 days at 37°C, 92% humidity and 5% CO₂ in air.

Results: FSH concentration of 100 mIU/ml showed increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates (p<0.0001). A significantly higher number of follicles showed mucified cumulus cells, attached to the oocytes when ovulation started within 16-24 hours post hCG (97 and 80%, respectively; p<0.0001). Successful ovulation failed to occur when the follicles were allowed to ovulate without hCG administration or more than 24 hours post hCG administration. While, in the medium containing FSH and 1.5 IU hCG, the ovulation percentage reached a maximum of 97% as compared to that seen for FSH-containing medium only (81%) or in control experiment (10%). So, the effect of FSH + hCG was highly significant over the control medium (p<0.0001).

Conclusion: Recombinant hCG and FSH are effective in promoting oocyte maturation in a clinical IVF program when administered in combination.

Key words: Follicle stimulating hormone, HCG, Preantral follicles, Oocyte maturation, GVBD.

P-25

Genetic aspects of male Infertility: Role of Y chromosome microdeletions and mutations in Androgen receptor and CFTR genes

Mirfakhraie R^{1,4}, Kalantar SM², Salsabili N³, Montazeri M⁴, Fazli H², Houshmand M⁴, Mirzajani F⁴.

1 Science and Research Branch, Islamic Azad University (IAU), Tehran, Iran.

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

3 Mirza Kouchak Khan Hospital, Tehran University of Medical Sciences, Tehran, Iran.

4 National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

E-mail: rmirfakhraie@nigeb.ac.ir

Introduction: Genetic factors cause about 10% of male infertility. Therefore in the present study the role of Y chromosome microdeletions; mutations and polymorphisms in CFTR and androgen receptor genes was investigated in patients with non obstructive azoospermia.

Materials and Methods: In the first section the existence and or absence of 8 sequence tagged sites markers representing AZF regions (AZFa, AZFb, AZFc and AZFd) was studied using Multiplex PCR. In the second step, the occurrence of common mutations and polymorphisms and also probable mutations in exons 4, 7, 10, 11, 20 and 21 of the CFTR gene was investigated using ARMS-PCR, PCR-RFLP, SSCP and TTGE methods. Finally, in the third step the incidence of Androgen receptor gene mutations and polymorphisms was studied using SSCP and Real-Time PCR methods among the patients and the control group which resulted in the detection of only P505T mutation in the first exon of AR gene in one of the patients which was not previously reported elsewhere.

Results: Of the total 106 azoospermic men analyzed, 14 individuals (13.21%) showed Y chromosome deletion, of which deletion in AZFb region was the most common (71.43%) followed by AZFc (50%), AZFd (42.86%) and AZFa (21.43%). None of these microdeletions was detected in the control fertile group. Also in the case of AZFc partial deletions, no significant statistical correlation was observed between the incidences of gr/gr, b1/b3 and b2/b3 deletions and male infertility (P values were 0.153, 0.465, and 0.447 respectively). 13 patients (21.26 %) showed 406-6T>C, A120T, I148T, ΔF508, G542X, L1304M and IVS8-5T mutations. To best of our knowledge this the first report about the mutation L1304M worldwide. No significant statistical correlation was observed between the incidences of Tn and M470V polymorphisms and male infertility (p-values were 0.738 and 0.755 respectively). Meanwhile the analysis of CAG repeats lengths

showed a significant statistical correlation between the patients and fertile controls (p=0.014).

Conclusion: Since most of the studied patients were candidates for assisted reproductive technologies such as ICSI and TESE, and considering the fact that genetic alterations may transmit to the off springs who are conceived with ICSI, our data support an important role for genetic factors, which have an important implication in the clinical evaluation of infertile men and in prediction of probable outcome of using assisted reproductive technologies and may help specialists for choosing the appropriate treatment method.

Key words: Y chromosome microdeletions, Male Infertility, CFTR genes.

P-26

Beneficial effect of luteal-phase GnRH agonist administration on implantation rate after ICSI

Ayazi Rozbahani M, Dehghani Firouzabadi R, Tabibnejad N.

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

E-mail: ayaziroz@yahoo.com

Introduction: To evaluate the effect of GnRH agonist administered in the luteal phase on ICSI outcome.

Materials and Methods: One hundred eighty women undergoing ovarian stimulation for ICSI were enrolled in this study. Patients were randomly assigned to receive a single dose of GnRH agonist or placebo. Implantation rate and clinical pregnancy rate were the main outcomes.

Results: Administration of 0.1 mg of GnRH agonist triptorelin on day 3 after embryo transfer led to a significant improvement of implantation rate (12.3% Vs.7.3%) and clinical pregnancy rate (25.5% Vs. 10%) as compared with placebo.

Conclusion: Luteal phase GnRH agonist administration enhances ICSI clinical outcomes

Key words: luteal phase support, GnRH agonist, ICSI.

P-27

Investigation of the methylation status of BAX CpG Island in the endometrium of patients with unexplained infertility

Shokri M, Noruzinia M, Abdul-Tehrani H, Javanmardi M, Keyhane M.

Tarbiat Modares University, Tehran, Iran.

E-mail: mehdi.shokri60@yahoo.com

Introduction: DNA methylation in CpG Islands of higher eukaryotes has important roles in epigenetic regulation of genome. Unexplained infertility is a diagnosis of exclusion, when the standard investigation of both the female and male partner has ruled out other infertility diagnoses. Apoptosis has been shown to be a pivotal regulator of endometrial function during the menstrual cycle and implantation. In this study, we investigated the role of aberrant DNA methylation in *BAX* gene promoter (which regulates spontaneous apoptosis) in unexplained infertile women.

Materials and Methods: The endometrial tissues were collected from 5 unexplained infertile and 5 fertile women. Methylation was examined by methylation specific PCR (MSP_PCR)

Results: Endometrial tissues in unexplained infertile women did show hypermethylation in *BAX* gene promoter compared with the fertile group.

Conclusion: Increased *BAX* gene promoter methylation ratio can influence infertility or vice versa.

Key words: Apoptosis, Unexplained, Infertility, Bax.

P-28

Effect of pomegranate juice (*Punica granatum L.*) consumption on sperm parameters and fertility potential in mice

Amini Rad O, Khalili MA, Miresmaeili SM.

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

E-mail: khalili59@hotmail.com

Introduction: Now-a-days, herbal drugs are used more often than before. Pomegranate juice (PJ) is among the fruits that has a high antioxidantal effect. There have been extensive studies about the effect of PJ on blood pressure, diabetes, Alzheimer disease, and others. Since, sperms are poor in combating with free radicals; this study was conducted to explore the antioxidantal effect of PJ on sperm parameters (count, morphology, motility) and fertility potential in mice.

Materials and Methods: 15 male mice were studied with regard to their sperm parameters and fertility potential. Sperms were categorized into three groups with regards to their motility: progressive, non-progressive, immotile. Morphology consisted of normal and abnormal sperms. Mice were divided into control group (n=5) and experimental group (n=10). The experimental group received 20% pomegranate juice for 1 month (duration of spermatogenesis is 1

month in mice). The control group had free access to water. We took one generation from each group to study the fertility rate. After killing the animals, a sample from the tail of epididymal region was taken to test the sperm parameters by light microscope.

Results: The results showed that PJ consumption increased sperm count (p=0.057), also significantly decreased non-progressive sperm motility (p=0.02), and improved progressive motility (P=0.06). In addition, the normal morphology of the experimental group improved significantly (p=0.008). The rates of fertility potential were noticed to increase from 5.8±4.8 in controls to 10.0±1.26 in experimental mice.

Conclusion: PJ is able to improve the quality of sperm parameters, as well as fertility potential in mice. Probably, intake of this antioxidant by infertile men improves the quality of their sperm parameters.

Key words: Pomegranate juice, Sperm, Fertility potential, Mouse.

P-29

Evaluation of semen analysis and the outcomes of IVF and ICSI cycles in cases with Polyzoospermia

Karimzade A¹, Hagimaghsoodi F², Khalili MA², Hosseiny Sharifabad M².

1 Department of Internal Medicine, University of Medical Sciences, Kerman, Iran.

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

E-mail: maghsoodi85@yahoo.com

Introduction: WHO suggested that men with sperm concentration of >250 million/ml are classified as Polyzoospermia. The incidence of polyzoospermia in the infertile male population ranges from 0.2% to 4.2%. According to many authors, this condition is associated with a reduced sperm motility and altered fertility performance. The aim of this study was twofolds: 1. to evaluate the semen parameters in polyzoospermic ejaculates in 94 cases; 2. to assess the outcome of IVF or ICSI cycles in 27 cases with polyzoospermia.

Materials and Methods: 121 polyzoospermic individual included in this study. Microscopic and macroscopic parameters of semen samples in 94 polyzoospermics who had no treatment (Group I) were evaluated using WHO criteria. 27 polyzoospermics with IVF or ICSI treatment (Group II) was also assessed. The data regarding the sperm

parameters, oocytes, fertilization rates, embryo development, and pregnancy rates were analyzed using t-test.

Results: The mean of sperm parameters were within normal range as classified by WHO. In group I, the mean of sperm progressive motility (fast and slow) was 15.05 +15.56 and 39.67 +15.45, respectively. Data also showed that in group II, from 234 retrieved MII oocytes, 144 oocytes fertilized (61.5%), 112 embryos were formed (77.7%), and 73 embryos were transferred (65.1%). 22 out of 27 patients did not get pregnant (infertile); while, the rest became pregnant, fertile, (18.5%). The etiological factors were: 5 cases with female factor, 8 with male factor, and 14 were considered with unexplained infertility. In 3 pregnant cases, the sperm morphology of their spouse was >30%.

Conclusion: Patients with Polyzoospermia have semen parameters within normal range. The elevation in sperm concentration (polyzoospermia) is not correlated with lower rates of fertilization or pregnancy in ART cycles.

Key words: Polyzoospermia, Male infertility, IVF, ICSI.

P-30

Comparison of survival rate and developmental competence of mouse MII oocyte after freezing by slow freezing and vitrification

Agha-rahimi A, Kalantar SM, Azarnia M, Soleimani M, Miresmaeili SM.

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

E-mail: smkalantar@yahoo.com

Introduction: Oocyte freezing is the only method to preserve the reproductive capacity for woman at risk of losing it because of premature ovarian failure, pelvic diseases, surgery, radiotherapy or chemotherapy treatments related to the ovary. But oocyte storage has faced technical difficulties compared with sperm or embryo cryopreservation because of specific structure of oocyte. The purpose of this study is investigated the morphological survival rate and developmental competence of thawed oocytes after cryopreservation by slow freezing or vitrification.

Materials and Methods: Female mice superovulated with intraperitoneal injection of PMSG and HCG. Ovulated MII mouse oocytes were allocated to the three groups as slow frozen, vitrified and control groups. Vitrification performed using ethylene glycol (EG) and

dimethyl sulfoxide (DMSO) and slow freezing using propanediol (PROH). After thawing the surviving MII oocytes in both cryopreserved and control group were inseminated for in vitro fertilization (IVF) and their developmental ability were compared.

Results: The survival rate of post thawed mature oocytes in vitrification group was 73.3% and in the slow freezing was 33.3%. Then following IVF, the percentage of cleaved embryos in control, vitrification and slow freezing groups was 64%, 25% and 20%, respectively.

Conclusion: The result showed that survival rate after thawing in vitrification was greater than slow freezing while the cleave embryo rate did not. Vitrification seems to be an effective, easy and rapid method for the cryopreservation of mouse MII oocytes.

Key words: Vitrification, Slow freezing, Survival rate, MII oocyte, In vitro fertilization.

P-31

Cyclophosphamide induced histological, histomorphometrical and histochemical changes in testis and, DNA damage and Chromatin abnormality in rat sperm

Ahmadi A, Resvanfar MA, Sadrkhanlou R, Abdollahi M.

Department of Embryology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

E-mail: dr.ahmadi2007@yahoo.com

Introduction: Cyclophosphamide (CP) is a chemotherapeutic agent used for the treatment of a variety of tumors. Cytotoxicity and gonadotoxicity are important for selection of treatment specially in young Hodgkines patients. The goal of this study was to determine the effects of CP on testicular dysfunction and spermatozoa fertility indices of male rat.

Material and Methods: 30 adult male Wistar rats were randomly divided into 3 groups (n=10 each) with two groups serving as control and sham (normal saline), in the drug treated group were gavaged 6 mg/kg/day cyclophosphamide for 30 days. All rats were sacrificed by CO₂ inhalation and testis tissues were removed and prepared for Histological (H&E, Vaigrate Iodine and Toloedine blue (for mast cells) staining) and Histochemical (by cryosection and Lipase, Oil Red O staining methods) study. In addition spermatozoa were removed from cauda epididymis and analyzed for sperm motility, concentration in the cauda epididymis, viability and sperm chromatin quality

and DNA integrity was assessed by Aniline blue and Acridine Orange staining following sperm sample preparation. Serum testosterone level was determined by radioimmunoassay and FRAP and TRAPS assays were used to determine total antioxidant power and lipid peroxidation respectively in testis tissues and plasma.

Results: This study confirmed that treated by CP had significant decrease the testosterone level in plasma. CP had significant increase the fat peroxidation level and decreases the total antioxidative capacity in the plasma and testis tissues. These changes were associated with significant increase in DNA Damage and chromatin abnormality in the cauda epididymal spermatozoa as evidenced by Acridine Orange (AO) and Aniline blue staining respectively. Treatment of male rat with CP caused significant decrease in sperm count, motility, and viability, while abnormal sperms increased as compared to control. Histological and histomorphometrical study confirmed that CP had significant decrease the somniferous tubules diameter, number of germinal cells, Tubule Differentiation Index (TDI) and spermatogenesis in the somniferous tubules. Toluidine blue staining confirmed that CP had significant increase the number of Mast cells in testis and epididymis tissues.

Conclusion: Histochemical study by Oil Red O and Lipase methods confirmed that CP had significant increase the fat droplets in the somniferous tubules and lipase reaction confirmed that the CP had increase the lipase enzyme in the testis tissue.

Key words: Cyclophosphamide, DNA Damage, Histochemical, Histomorphometrical, Acridine orange, Testis.

P-32

Dietary Antioxidant intake and male infertility

Nadjarzadeh A¹, Mostafavi E², Seraji A², Shidfar F¹, Chamari M³.

1 Faculty of Health, Iran University of Medical Sciences, Tehran, Iran.

2 Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

3 Faculty of Health, Tehran University of Medical Sciences, Tehran, Iran.

E-mail: azadehnajarzadeh@gmail.com

Introduction: About 15% percent of all couples in the world and 25% of couples in Iran experience infertility in their reproductive years. Nearly 25% of infertility among couples can be attributed to semen quality and other male factors. Its etiology

is poorly understood, although some factors such as antioxidant intake have been implicated. The purpose of the present study was to determine and comparison of antioxidant vitamins intake (Vit C, E and carotenoides) in fertile and infertile men.

Materials and Methods: This study was conducted in infertility clinic in Mirza koochak khan hospital, Tehran. 32 idiopathic infertile men with abnormal semen analysis and 22 healthy donors were recruited. Average daily nutrient intake was derived from semi-quantitative food frequency questionnaire. Semen analysis was done according to WHO criteria. Diet analysis was performed using the Food Processor software. Statistical analysis was performed using the SPSS ver.12 software. Results are reported as mean±SD. Differences among the cases and controls were evaluated by t-test.

Results: The intake of vitamin C and carotenoides in two groups were not different. But the intake of vitamin E was significantly lower in infertile than those of fertile men. (10.9±6.2 VS. 14.5±3.8 p<0.02). Also, 82 percent of infertile men and 17.6 percent of fertile men have less than 75% of RDA (Recommended Dietary Allowances) of Vitamin C (p<0.04).

Conclusion: The intake of some of the antioxidant vitamins are low infertile men with idiopathic semen abnormalities. It could be effect on the fertility potential.

Key words: Vitamin, Infertile men, Antioxidant intake.

P-33

A comparison study of zinc and folate intake in relation to male infertility

Nadjarzadeh A¹, Mehrsai AR², Mostafavi E², Amirifard S².

1 Faculty of Health, Iran University of Medical Sciences, Tehran, Iran.

2 Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

E-mail: azadehnajarzadeh@gmail.com

Introduction: About 15% percent of all couples in the world and 25% of couples in Iran experience infertility in their reproductive years. Oxidative stress may play a role in male factor sub fertility. Both folate and zinc have antioxidant properties that counteract reactive oxygen species (ROS). The purpose of this study was to determine and comparison of these two antioxidants between sub fertile men with abnormal semen parameters and healthy donors.

Materials and Methods: This study was conducted in infertility clinic in Mirza koochak khan hospital, Tehran. 32 idiopathic infertile men with abnormal semen analysis and 22 healthy donors were recruited. Average daily nutrient intake was derived from semi-quantitative food frequency questionnaire. Semen analysis was done according to WHO criteria. Diet analysis was performed using the Food Processor software. Statistical analysis was performed using the SPSS ver.12 software. Results are reported as mean±SD. Differences among the cases and controls were evaluated by t-test.

Results: Intake of zinc of 84.4 percent of infertile men and 13.6 percent of controls were deficient (less than 75% of recommend dietary Allowances). The mean intake of zinc and folate were significantly different in two groups ($p<0.000$).

Conclusion: The intake of zinc and folate in idiopathic sub fertile men with abnormal semen characteristics are less than those of healthy men. These two micronutrients may have some effects on fertility potential.

Key words: Zinc, Folate Intake, Male infertility.

P-34

The correlation between reactive oxygen species and leukocytes in normozoospermia and oligoasthenoteratozoospermia ejaculates

Rahavi A, Ali-Haidari A, Khalili MA, Ghasemzadeh J, Tabibnejad N.

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

E-mail: rahavi6381@gmail.com

Introduction: An important factor involved in infertility is reactive oxygen species (ROS). ROS can damage sperm DNA, and involve in lipid peroxidation. ROS elevation is under the influence of leukocyte activation. The objective of this prospective study was to evaluate the level of ROS as well as leukocyte in normozoospermic (NO) and oligoasthenoteratozoospermic (OAT) ejaculates.

Materials and Methods: The study population consisted of 75 individuals who were referred to research and clinical center for infertility in Yazd for semen analysis. 50 out of 75 men were NO, and the rest were OAT. ROS was measured with laminator, while leukocyte concentration was done with ENDTZ test.

Results: The results showed that ROS level in OAT was significantly higher compared with NO

(1253.49 ± 200.95 vs. 75.64 ± 149.52 ; $p=0.00$). Also, men with OAT were divided into 2 groups with sperm morphology and motility $>5\%$ and $<5\%$. In group $>5\%$, ROS level was significantly higher than group with $<5\%$ (3627.55 ± 407.79 vs. 81.29 ± 100.48 ; $p=0.007$). In addition, leukocyte concentration in NO was $0.07\pm 0.22\times 10^6$; while, it was $0.12\pm 0.20\times 10^6$ in OAT samples; $p=0.35$).

Conclusion: The results indicate that although ROS is present in normal seminal samples, but it is significantly higher in OAT. This shows the vital role of antioxidants, which may improve the sperm quality. Further clinical studies will pinpoint the antioxidant capacity in improving the seminal contents.

Key words: Infertility, OAT, ROS, Leukocyte, Sperm parameters.

P-35

Comparing of colony formation of spermatogonial stem cells of adult mice after co-culture with sertoli and STO cells

Mohammadi SM, Movahedin M.

Department of Anatomy, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.

E-mail: mmohamady2006@yahoo.com

Introduction: Although spermatogenesis is essential for reproduction, little is known about spermatogonial stem cells (SSCs). These cells provide the basis for spermatogenesis throughout adult life by undergoing self-renewal and providing progeny that differentiate into spermatozoa. Sertoli cells in the seminiferous tubules that closely interact with germ cells to create a favourable environment for spermatogenesis. The aim of this study was to evaluate the effect of sertoli and STO feeder layer cells on colony formation of SSCs during two weeks period. $\beta 1$ and $\alpha 6$ mRNA expression were evaluated by RT-PCR.

Materials and Methods: At the first step sertoli and SSC were isolated from adult mice testis using two step enzymatic digestions and lectin immobilization. Characteristic of isolated cells was confirmed by immunocytochemistry with Oct4 and C-kit for SSC and vimentin for sertoli cells. SSCs were cultured on the top of the sertoli and STO feeder layers for two weeks. The number and diameter of colonies were evaluated by invert microscope during these two weeks. $\beta 1$ and $\alpha 6$ mRNA expression were assessed using RT-PCR.

Results: The results of repeated measures ANOVA and paired samples t-test as post hoc

showed significant difference between four time points, separately in sertoli, STO and control groups for both of the number and diameter of colonies ($p < 0.05$). But in STO and control groups, the time points of 10th and 14th days were not significantly different ($p > 0.05$). Also, the results of one-way ANOVA and Tukey post hoc test showed that there was a significant difference between three groups, separately in each time ($p < 0.05$), and sertoli had higher mean in number and diameter, with respect to other two groups ($p < 0.05$). The results of RT-PCR showed that $\beta 1$ was expressed in three groups after two weeks culture but $\alpha 6$ was not expressed.

Conclusion: Based on the optimal effect of sertoli cells on spermatogonial stem cells in a co-culture system, as confirmed by some other studies, it is suggested use these cells for better colonization of SSCs.

Key words: *Spermatogonial stem cells, Adult mice, Sertoli, STO cells.*

P-36

Comparison of mature and immature sertoli cell co-culture on maintenance of spermatogonial stem cells in vitro

Tavakoli F^{1,2}, Shahverdi A¹, Pirouz M¹, Koruji M³, Shakeri M⁴, Baharvand H^{1,2}.

1 Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran.

2 Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran.

3 Department of Basic Science, University of Social Welfare and Rehabilitation Science, Tehran, Iran.

4 Department of Animal Science, Agricultural Campus, University of Tehran, Tehran, Iran.

E-mail: f.tavakolifar_516@yahoo.com

Introduction: Spermatogonial stem cells (SSCs) are unique population of adult stem cells in mammalian testes which continuously provide gametes and transfer genetic material to the next generation. Various feeder layers have been tested to support the in vitro culture of SSCs; however, age effect of feeder cells has been remained a controversial issue. This study was initiated to compare Sertoli cells derived from neonatal and adult mice to examine age effect of Sertoli cells in the maintenance of mouse SSCs in vitro.

Materials and Methods: SSCs were isolated from testes of 6 day-old mice and culture in vitro at the presence of Glial-derived neurotrophic factor (GDNF) for 10 days and

then transferred to Sertoli cells isolated by DSA lectin from neonatal (6 day-old) and adult (6-8 week-old) mice. After 5 days, diameter and number of SSC colonies were measured. Immunostaining was used to detect expression of spermatogonial markers including $\alpha 6$ -Integrin, PLZF, C-kit and Oct-4. In addition, SSC colonies were harvested at day 5 and the percentage of $\alpha 6$ -Integrin-positive cells was measured by flowcytometry. Transplantation assay was used to confirm the stemness of spermatogonial cells at the beginning of the study.

Results: Immunostaining analysis showed that our culture system contained SSC colonies as they were positive for $\alpha 6$ -Integrin, PLZF and Oct-4 and negative for C-kit. In addition, these stem cells were functional as they were able to migrate to seminiferous basal membrane after transplantation to testes of busulfan-induced infertile adult mice. Results showed 5 days after co-culture of SSCs with Sertoli cells, the diameter of colonies was significantly higher on neonatal Sertoli cells even though there was no significant difference in the number of colonies. Flowcytometry analysis revealed that there was significant increase in the number of $\alpha 6$ -Integrin-positive cells in the culture with neonatal Sertoli cells ($61.40 \pm 5.60\%$) in contrast to culture with adult Sertoli cells ($29.56 \pm 6.59\%$).

Conclusion: Based on our results, the number of SSCs was higher in co-culture with neonatal Sertoli cells. It could be referred to difference in microenvironments that neonatal and adult Sertoli cells provide for in vitro culture of SSCs. As SSCs was derived from neonatal mice, another interpretation is that more suitable culture condition could be obtained when SSCs and feeder Sertoli cells are derived from mice in the same age; either from neonatal mice or from adult ones. Further studies would confirm these hypotheses.

Key words: *Sertoli cell co-culture, Spermatogonial stem cells, In vitro.*

P-37

Embryonic stem cell sphere: A controlled method for production of mouse embryonic stem cell aggregates for differentiation

Rohani L^{1,2}, Karbalaie Kh¹, Vahdati A², Hatami M³, Nasr Esfahani MH¹, Baharvand H³.

1 Department of Stem Cells, Royan Institute, Esfahan Campus, Esfahan, Iran.

2 Department of Biology, Esfahan University, Esfahan, Iran.

3 Department of Stem Cells, Royan Institute, Tehran, Iran.

4 Department of Stem Cells, Royan Institute, P.O. Box: 19395-4644, Tehran, Iran.

E-mail: Baharvand50@yahoo.com

Introduction: Embryonic stem cells (ESCs) are of significant interest as a renewable source of non-proliferating cells.

Materials and Methods: Differentiation of ESCs is initiated by the formation of embryoid bodies (EB). Standard methods of EB formation are limited in their production capacity, the variation in EB size and formation of EB through frequent passages. Here we have reported the utility of microencapsulation technique for overcoming aforementioned limitations by mass production of mouse ESCs in alginate beads called ESC spheres.

Results: Analysis of encapsulated ESC spheres by flow cytometry showed the similar percent of Oct-4 and SSEA-1 expression in comparison with routine culture of ESCs. Moreover, the ESC spheres showed the ability of differentiation.

Conclusion: The results demonstrated that alginate encapsulation as a simple bioreactor, provides a scalable system for mass undifferentiated ESC sphere production with similar sizes and without the need of frequent passages for differentiation, clinical and pharmaceutical applications.

Key words: Embryonic stem cell, Embryoid bodies, Mouse.

P-38

Comparison of negative immunodepletion and bFGF effect on isolation of mesenchymal stem cells from umbilical cord blood

Vasaghi A, Deghani A, Khademolhosseini M, Attar A, Khosravi Maharlooei M, Monabati A.

Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran.

E-mail: atieh_va@yahoo.com

Introduction: It is well accepted that mesenchymal stem cells (MSC) are promising populations for new clinical concepts. Although Bone marrow (BM) is the most common source of MSCs, aspirating BM is an invasive procedure and the number of cells and their differentiation potential decreases with age. Therefore, umbilical cord blood (UCB) was introduced as an alternative source of MSCs. However, previous studies showed a success rate of 30% or even complete failure in isolation of MSCs from UCB using

regular methods. In present study we investigated the effectiveness of bFGF and negative immunodepletion (NI) and combination of both for isolating and expansion of MSCs.

Materials and Methods: UCB was collected with informed consent after normal vaginal delivery. Each sample was divided into 4 groups. NI was applied for groups A and B. Cells in groups B and C were cultured in media supplemented with 10ng/ml bFGF, while group D was taken as control. We compared MSCs of these groups regarding morphology, the success rate of isolation and multiple differentiation potential.

Results: By NI approximately one third of the cells were depleted. Many of the cells became spindle-shaped after one week. In group C and D population had heterogeneous morphology in shape and size. But three week after initial seeding cells showed rounded shape. By the fourth week many of them died in all groups and apoptotic cell bodies remained.

Conclusion: The results of our study show that neither bFGF nor NI guarantee isolation of MSCs from UCB. Therefore further critical parameters should be investigated.

Key words: Umbilical cord blood, Mesenchymal stem cells, negative immunodepletion, bFGF.

P-39

Actinidin a new collagenase for isolation and primary culture of thymic epithelial cells from rat thymus

Shirvani Farsani Z.

Department of Biology, Razi University, Kermanshah, Iran.

E-mail: zbshirvani@gmail.com

Introduction: Proteolytic enzymes, specially collagenase, are used to digest extracellular matrix, cells isolation and primary culture. It is important to find new sources of plant or animal protease instead of bacterial or tissue collagenase. In the present research, actinidin, a plentiful protease in kiwifruit (*Actinidin deliciosa*), was used for isolation and culture of cells from thymic epithelial cells (TEC) from rat thymus.

Materials and Methods: The thymus was taken out. The gland was minced into small pieces and suspended in the PBS containing 1, 2, 4, 8, or 16 mg/ml actinidin for 1, 2, 3, or 4 h with gentle shaking. The cell pellet was resuspended in William's E culture medium. The cell suspensions were cultured in dishes precoated with collagen.

Results: Rat TEC was properly isolated after digestion of thymus in 4 mg/ml actinidin for 4 h at 37 °C. The isolated cells were adhered to collagen precoated dishes after washing. After 24 h of culture, the adherent cells were flattened and showed polygonal morphology with small nuclei. The viability of the cells as judged by the trypan blue test was estimated to be 90–95% in all isolations.

Conclusion: The results showed that actinidin has not toxic effect on separated cells and is a novel and suitable protease for isolation of rat TEC.

Key words: Actinidin, Collagenase, Kiwifruit, Thymic epithelial cells.

P-40

Comparing the neonatal growth index in unwanted and wanted pregnancies

Araban M, Khasaeiyan S, Javanmardi M, Karimian Z, Bahrami N.

Nursing and Midwifery Faculty, Shahid Sadoughi University of Medical Science, Yazd, Iran.

E-mail: araban62@ssu.ac.ir

Introduction: One of health difficulties in developing countries threatened health, Is unwanted pregnancy outcomes which has bad effects on reproductive health. Studies showed that mother paying attention to unwanted pregnancy decreases that can affect fetal growth. The Purpose of this cross sectional study was determining the comparison of unwanted and wanted pregnancy outcomes.

Materials and Methods: 225 women referring to Tehran selected hospitals in 2007 were selected. An information form was used to collect data. Its validity and reliability were achieved by content and test-retest methods respectively. 65 cases were assigned to unwanted group and 165 cases to wanted pregnancy group. Pregnancy outcome: birth weight, length, head and chest circumference and apgar score were investigated. Data collection was done by multi stage sampling method. Data analysis was done by SPSS 15 and t test and χ^2 test.

Results: Mean age was 25.86 ± 4.6 and mean gravity was 1/7. There was a significant association between unwanted pregnancy and head circumference, $p < 0.05$. Although there was no significant association between unwanted pregnancy and the other outcomes, these outcomes were poorer in the unwanted pregnancy. Also women in unwanted groups had less weight gain during pregnancy.

Conclusion: With regards to these results that shows poorer neonatal growth indexes in unwanted pregnancy, and then doing effective action for decreasing unwanted pregnancy and approaching to unwanted pregnancy as a high risk pregnancy is necessary for prevention of bad effects of unwanted pregnancy on society health.

Key words: Unwanted pregnancy, Outcome

P-41

Effect of lead on proliferation and neural differentiation of mouse bone marrow-mesenchymal stem cells

Kermani Sh^{1,2}, Karbalaie Kh¹, Madani SM², Jahangirnejad AA², Bogheban Eslaminejad MR², Nasr Esfahani MH¹, Baharvand H^{3,4}.

1 Department of Stem Cells, Royan Institute, Esfahan Campus, Esfahan, Iran.

2 Department of Biology, Esfahan University, Esfahan, Iran.

3 Department of Stem Cells, Royan Institute, Tehran, Iran.

4 Department of Developmental Biology, University of Science and Culture, Tehran, Iran.

E-mail: Baharvand50@yahoo.com

Introduction: Bone marrow-mesenchymal stem cells (MSCs) are considered to be an ideal source of stem cells for assessing the effects of environmental toxins on the proliferation, multipotency and differentiation of adult stem cells. The aim of this study was to investigate the effect of lead on the proliferation and neuronal differentiation of murine MSCs.

Materials and Methods: MTT assay used in this study revealed that while the proliferation of MSCs is sensitive to higher than 10 μ M lead, a 50% reduction in the rate of their proliferation can be achieved in the presence of 60 μ M lead.

Results: The results of immunocytochemistry and RT-PCR showed that β -mercaptoethanol induced-neuronal differentiation is also reduced after the treatment of MSCs by 60 μ M lead. Furthermore, the Comet assay analysis of MSCs showed a substantial increase in DNA damage in the lead treated cells compared to the control.

Conclusion: Our results revealed for the first time that lead is not only cytotoxic to the survival and proliferation of MSCs but also inhibits their differentiation to neurons in a dose-dependant manner. Therefore, MSCs appear to be an alternative method for assessing the cytotoxic effects of such environmental hazards.

Key words: Lead, Mesenchymal stem cells, Neural differentiation, Proliferation.

P-42

Spermatogonial transplantation and subsequent orchidopexy in bilateral cryptorchid mouse model

Absalan F¹, Movahedin M¹, Mowla SJ².

1 Department of Anatomy, Tarbiat Modares University, Tehran, Iran.

2 Department of Genetics, Tarbiat Modares University, Tehran, Iran.

E-mail: forouzan_absalan@yahoo.com

Introduction: The mainstay of therapy for undescended testes is operative treatment. Surgery for cryptorchidism is important before germ cells become scarce; because infertility is associated with the lack of germ cells. The aim of this study was to examine the effect of spermatogonial transplantation and subsequent orchidopexy after creation of degenerative changes in bilateral cryptorchid mouse model.

Materials and Methods: Bilateral cryptorchidism was induced in immature mouse by returning two testes to the abdominal cavity via a surgical procedure. Orchidopexy and spermatogonial transplantation was performed 3 months after heat exposure. The number of spermatogonia and differentiated germ cell were measured.

Results: Transplantation of spermatogonial stem cells in to the mouse seminiferous tubules was successful in recipients which had severe tubular degeneration after induction of cryptorchidism. After transplantation of spermatogonial stem cell in to the cryptorchid testis, germ cell colonization was shown, the number of spermatogonia, spermatocytes returned to near normal range but spermatogenesis was recovered partially at the late stages of spermatogenesis.

Conclusion: The reason of partially return of spermatozoa after transplantation maybe is the severe effects of surgery on the blood supply or innervations of the testis and a serious sac was not reconstructed around the testis without adhesions, which may be important for normal spermatogenesis.

Key words: Cryptorchidism, Mouse, Spermatogonia, Testis, Transplantation.

P-43

The study of general health of infertile couples in Yazd 2008

Soltani HR, Vaziri S, Dehestani M, Moghimi M, Zare A.

Medical School, Islamic Azad University, Yazd Branch, Yazd, Iran.

E-mail: hrsrmed@yahoo.com

Introduction: To identify the predictive factors of mental disorders in patients with suspected infertility.

Materials and Methods: The patients admitted to the Infertility Service of Madar hospital of Yazd for their infertility problems were studied. The sociodemographic and clinical variables were obtained by GHQ-28 questionnaire and the clinical records of the patient. The GHQ-28 questionnaire was used to assess the status of mental health, and a score of ≥ 6 was taken as the cut-off point for being a 'probable psychiatric case'. Crude odds ratios (OR \pm 95% CI), and adjusted OR according to a logistic model, were calculated in order to study the variables associated with the mental disorders. The tabulation and analysis of data was carried out with the SPSS v.14 (for Windows) program.

Results: From September 2008 to December 2008, 200 patients were studied; 72% were female and the mean age was 23.6 years (SD=2.4). 49.2% of the patients were scored at ≥ 6 on the GHQ-28, and the sub-scale accounting for the highest scores was that of somatic symptoms of psychological origin. The adjusted ORs showed that female sex (OR: 2.5; 95% CI: 1.2–5.0), infertility type (OR: 2.3; 95% CI: 1.1–4.4), education level (OR: 2.0; 95% CI: 1.1–3.9), were the predictive variables for being a 'probable psychiatric case', whereas neither age nor co morbidity were predictive.

Conclusion: The percentage of subjects considered to be a 'probable psychiatric case' among patients with suspected infertility was high. Screening for mental disorders and primary diagnosis associated are measures recommended in these patients.

Key words: Mental health, Infertility, Yazd.

P-44

The effect of serum and follicular fluid Anti-mullerian hormone levels on in vitro fertilization outcomes in normal and polycystic ovary syndrome women

Hossein G¹, Arabzadeh S¹, Rashidi B², Agha Hosseini M³, Zeraati H⁴.

1 School of Biology, University College of Science, University of Tehran, Tehran, Iran.

2 Imam Khomeini Hospital, Vali-e-Asr Reproductive Health Research Center, School of Medicine, Tehran University of Medical Science, Tehran, Iran.

3 Shariati University Hospital IVF Center, School of Medicine, Tehran University of Medical Science, Tehran, Iran.

4 Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

E-mail: bhrashidi@yahoo.com

Introduction: It has been shown that high levels of serum anti-mullerian hormone (AMH) is associated with polycystic ovary syndrome (PCOS). This study investigates the relationship between serum or follicular fluid (FF) AMH concentrations and In Vitro Fertilization (IVF) outcomes in normal and PCOS women.

Materials and Methods: 33 Women with PCOS, (20-47) years, and 56 normal women, (21-42) years, were recruited from Vali-e-Asr Reproductive Health Research center and Shariati University Hospital IVF center. On the cycle day 3, serum samples and on the day of oocyte retrieval FF from preovulatory follicles was collected for AMH measurements by Elisa method.

Results: Median (range) serum AMH level (ng/ml) was markedly increased in the PCOS group [15.06 (0.10-50.70) vs. 3.38 (0.42-9.91) in normal; $p < 0.001$]. In both groups, serum AMH levels showed a significant positive correlation with oocyte number ($r = 0.40$; $p < 0.01$ vs. $r = 0.49$; $p < 0.01$ in PCOS) and oocyte maturation ($r = 0.46$; $p < 0.001$ vs. $r = 0.59$; $p < 0.001$ in PCOS). Regarding FF AMH levels (ng/ml), median (range) was higher in PCOS group [8.21 (0.39-127.59) vs. 2.13 (0.62-13.69) in normal; $p < 0.05$]. The FF AMH levels showed a positive correlation with implantation ($r = 0.32$, $p < 0.05$) and biochemical pregnancy ($p < 0.05$) rates in normal but not in PCOS.

Conclusion: Concentration of AMH in serum, but not in FF, may constitute a useful marker of oocyte number and oocyte maturation in PCOS and normal group. While, AMH levels in FF could be a predictive factor for implantation and biochemical pregnancy rates only in normal group.

Key words Anti-mullerian Hormone/AMH, Polycystic ovary syndrome/PCOS, IVF outcomes.

P-45

Evaluation of premature ovarian failure following cauterization in Polycystic Ovary Syndrome

Moridi I¹, Fallahian M¹, Saremi A², Mahdian M¹.

1 Department of Obstetrics and Gynecology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2 Department of Obstetrics and Gynecology, Infertility Center, Sarem Hospital, Tehran, Iran.

E-mail: ir_moridi@yahoo.com

Introduction: Polycystic ovary syndrome (PCOS) is a frequent hormonal complication in women which is predominantly diagnosed with chronic anovulation and hyperandrogenism. Laparoscopic Ovarian Drilling (LOD) is considered as an effective means of treating Clomiphene-resistant anovulatory infertile patients with PCOS. To evaluate the efficacy of cauterization on ovarian failure, endocrinological parameters, ovarian volume and patient related outcomes.

Materials and Methods: Sixty five clomiphene-resistant anovulatory patients with PCOS have participated in this retrospective single blind clinical trial. They were randomly selected and divided into case and control groups and were asked to take the routine laboratory tests for endocrinologic assessment and to undergo a sonographic imaging for ovarian evaluations. The data were recorded and analyzed using group t-test and chi-square.

Results: There was no significant difference between the post operative levels of FSH among the two groups ($p < 0.05$). However, the level of LH decreased significantly in the case group following LOD compared to the control group ($p < 0.05$). The ovarian volume also showed a significant decrease in the case group compared to the control group ($p < 0.05$) and finally the menstruation cycles happened more regularly and the pregnancy rate increased in patients who underwent LOD.

Conclusion: The results suggest that LOD could be an effective means of treating PCOS in clomiphene-resistant patients without increasing the risk of premature ovarian failure.

Key words: Polycystic Ovary Syndrome, Laparoscopic Ovarian Drilling, Premature Ovarian Failure.

P-46

Effect of chromium picolinate on insulin resistance and hormone profile in women with polycystic ovary syndrome

Moridi I, Salehpour S, Mahdian M.

Department of Obstetrics and Gynecology, (IRHRC), Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

E-mail: ir_moridi@yahoo.com

Introduction: Polycystic Ovary Syndrome (PCO) is the most common endocrine disorder in reproductive-age women, which is predominantly diagnosed with chronic anovulation and hyperandrogenism. These patients face increased risk of developing type2 diabetes and dyslipidemia. Unfortunately metformin and thiazolidinediones could have adverse effect influencing tolerance and compliance. Therefore, it would be ideal to find an effective insulin sensitizer, with few adverse effects that could be used long-term. Chromium is an essential element that plays a role in glucose and insulin homeostasis. To evaluate the efficacy of chromium picolinate on insulin resistance and hormone profile in women with polycystic ovary syndrome.

Materials and Methods: Fifteen patients with established PCOS will be treated with oral Chromium picolinate (800µg/d) for a period of 6 weeks. Hormone profile, lipid profile, fasting glucose, fasting insulin, 2HPP, post test insulin and other clinical measurements shall be recorded before and after the treatment phase. The data will be submitted to chi-square and fisher's test for analysis.

Results: This project is currently being conducted in the Infertility Research Center of Taleghani Hospital and the results will be reported by April 2009.

Conclusion: The initial phase of this project indicated an acceptable compliance of patients. We shall provide a definite conclusion by April 2009.

Key words: Polycystic Ovary Syndrome, Chromium picolinate, Hormone profile.

P-47

Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing IVF/ICS

Ayazi Rouzbahani M, Karimzadeh MA, Tabibnejad N.

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

E-mail: ayaziroz@yahoo.com

Introduction: To evaluate the influence of endometrial biopsy on increasing implantation rate in patients with recurrent implantation failures.

Materials and Methods: In a randomized control trial study, 93 women with at least two implantation failures were evaluated. In case group endometrial biopsy was obtained from 48 patients in the luteal phase of previous cycle and

implantation and clinical pregnancy rate were compared with 45 patients in control group.

Results: The implantation rate was determined as 10.9% in biopsy group compared with 3.38% in controls. Clinical pregnancy rate was significantly higher in case group than controls (27.1% and 8.9% respectively).

Conclusion: The results suggest that in vitro fertilization or intracytoplasmic sperm injection after endometrial biopsy increase pregnancy outcome.

Key words: Endometrial biopsy, IVF/ICSI, Pregnancy outcome, Recurrent implantation failure.

P-48

Comparison between GnRH agonist long protocol and GnRH antagonist protocol in outcome of the first cycle ART

Ahmadi Sh, Oskouian H, Dehghani Firouzabadi R.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medicine Sciences, Yazd, Iran.

E-mail: AHMADISHAHNAZ2005@yahoo.com

Introduction: Literature suggests that GnRH antagonists are comparatively more often used in cycles which have an unfavorable prior prognosis, and this protocol is an ideal one for poor responder patients. Up to now, however not enough prospective have been published to prove any beneficial effect of antagonists on the first cycle assisted reproductive technique. This prospective study was done to evaluate the efficacy of gonadotropin releasing hormone antagonist in comparison with the standard long protocol in the first cycle of ART.

Materials and Methods: We randomized 140 patients undergoing ART for the first time. Group 1 (n=70) was stimulated with a standard long protocol and group 2 (n=70) stimulated with GnRH antagonist.

Results : There was no statistically significant difference in the age, infertility cause, basal FSH, BMI, the number of oocytes retrieved, The number of M2 oocytes, embryo obtained, endometrial thickness and ovarian hyperstimulation syndrome in the two groups. But Serum estradiol and consumption of gonadotropins were significantly lower in the antagonist protocol. Cancellation rate due to poor-quality embryo in the antagonist protocol was higher but there was no significant

difference in the clinical pregnancy between the two groups.

Conclusion: GnRH-antagonist is an effective, safe, and well tolerated alternative to agonist in the first cycle of ART.

Key words: GnRH antagonist, GnRH agonist, Pregnancy rate, ART, Ovarian stimulation.

P-49

Growth characteristics analysis of the mesenchymal stem cells

Khoshkhou S, Yazdizade A, Attar A, khosravi Maharlooie M, Pourhabibi Zarandi N, Solhjoui Zh, Ahrari I, Monabati A.

Faculty of Medicine, Shiraz University of Medical Science, Shiraz, Iran.

E-mail:khoshkhou.sara@yahoo.com

Introduction: Determination of growth characteristics of different cell types is considered as an important way to study the effect of extracellular molecules such as different culture medias in vitro. A normal growth curve consists of three phases: 1-the lag phase is the time after subculture and reseeded in which cells adapt with new conditions, 2-the log phase is the period of exponential increase in cell number and 3-the plateau phase in which cell growth is reduced due to contact inhibition and depletion of nutrients. In this study we analyzed these parameters for mesenchymal stem cells. These data will be helpful for researchers who want to study effect of different culture medias and serums efficacy.

Materials and Methods: Mesenchymal stem cells were harvested from a culture plate, counted and brought into suspensions of 1×10^5 cells/ml (concentration A), 3×10^4 cells/ml (concentration B) and 1×10^4 cells/ml (concentration C), in 25 ml of media for each concentration. One milliliter (ml) of each was seeded in an arrow of two 24 well plates. Plates were incubated at 37°C in a humid atmosphere with 5% CO₂. Media exchange was done every three days. Every day one well of each concentration was trypsinized and cells were counted with a hemocytometer. Finally growth curve was drawn and parameters were calculated.

Results: Lag time was determined as more than one day, 4 days and 4 days, with log durations of 3 days, 4 days and 4 days for concentrations A and B and C respectively. Population doubling time was calculated by the formula " $(\log(N1) - \log(N0)) / \log 2$ " and results were 1.6, 1.12 and 1.53 for concentrations A, B and C.

Conclusion: Growth curve contained all three phases indicating that higher seeded cell number results in a lesser lag time and also a lower log duration, but lower cell concentrations lead to higher rates of proliferation. These data will be useful in understanding the effect of different culture medias, various growth factors and drugs on the growth characteristics of mesenchymal stem cells.

Key words: Mesenchymal stem cell, Stem cell growth characteristics, Population doubling time.

P-50

The effect of thyme (thymus vulgaris) decoction on pregnancy in rats

Zeinali F¹, Anvari M², Dashti MH³, Bioki SMH⁴.

1 Medical School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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

3 Department of Physiology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4 Pharmaceutical School, Isfahan University of Medical Sciences, Isfahan, Iran.

E-mail: moanvari@yahoo.com

Introduction: Thyme is a plant in Labiata family which different species of this herb affects the menstrual cycle and therefore is avoided in high doses especially during the gestational period. The aim of this study was assessing the effect of thyme Decoction consumption in 2nd gestational week on placenta and fetus in rats.

Materials and Methods: In this experimental study 10 pregnant Wistar rats weighting between 250-300 gr were randomly divided into 2 equal groups. Breeding of animals were done and the day on which a vaginal plug appeared was considered as the 1st day of gestation. The pregnant rats were acclimatized and fed in the same conditions during the gestational period. Animals in control group received tape water but the animals in test group received thyme Decoction in 2nd week of gestation. All animals were anesthetized and the fetuses were extracted through a cesarean section on 20th day of pregnancy. The uterus was excised, the placenta was removed and weighed, the number and placement of implantation sites, the number of live, dead or reabsorbed fetuses were recorded in each animal. All fetuses were weighed and stereomicroscopically examined for any morphological abnormalities in limbs, vertebral column, or head. Fetal height, fetal biparietal and placental diameters were also precisely measured.

Results: There were no significant differences in the number of live fetuses, between the test and control groups. No dead or reabsorbed fetuses and no morphological abnormalities were observed in live fetuses. The live fetuses in both groups showed the same weight, height and biparietal diameter but the length of tail in test group was longer than control group ($p < 0.05$). The placental weight was approximately identical in tow groups but its diameter in test group was smaller than the control group ($p < 0.05$).

Conclusion: according to our findings, although the thyme Decoction consumption in 2nd gestational week did not lead to any abortion or significant fetal anomaly, due to its adverse effect on tail length and placental diameter, it is recommended that the thyme consumption be with precaution especially during the pregnancy.

Key words: *Thymus Vulgaris, Abortion, Pregnancy, Placenta.*

P-51

Spermatogenesis and sperm morphological abnormalities

Ghasemzadeh J, Soleimani M, Gheisari H, Motamedzadeh L, Fazli H, Abdoli AM, Miresmaeili SM.

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

E-mail: jalal.1359@yahoo.com

Male reproductive system includes internal and external genitalia. External genitalia include scrotum and penis. Each testis is an oval structure about 4.5 to 5.1cm long and 3cm in diameter and located in the scrotum. each testis contains seminiferous tubules which are long, highly coiled, and looped tubules. Sperm are produced within the seminiferous tubules. Sertoli cells which are located within seminiferous tubules feed and protect developing sperms. Epididymis is a long tube located along the superior and posterior margins of the testes. Its function is sperm transport, storage and maturation (motility and fertility maturation). Sperm transport through the human epididymis last from 2 to 12 days. Process of sperm production is called "spermatogenesis" which begins at puberty and takes approximately 64 days in the human. The conversion of spermatids into mature spermatozoa is called "Spermiogenesis". The mature and normal sperm cell is approximately 60 μm in length and has a head, neck, middle piece and tail. The head should be oval in shape and 4 to 5 μm in length. The midpiece is slender and less than 1 μm in width.

The tail should be straight, uniform and thinner than midpiece, and approximately 45 μm long. Sperm morphology classification has a predictive value in invitro sperm fertilization. The following defects has been recognize according to WHO criteria:

(a) Head defects including large, small, tapered, pin, pyriform, round, amorphous, vacuolated heads and heads with small or large acrosomal area

(b) Neck & Midpiece defects including bent neck, asymmetrical insertion, thick and thin midpiece and cytoplasmic droplet.

(c) Tail defects including short, broken, bent, double, coiled tails and tail-tip coiling.

Key words: *Spermatogenesis, Testis, Sperm morphology.*

P-52

The Study of effective factors in unintended pregnancies in patients referring to the Sh. Kargar Hospital of Yazd, Iran

Soltani HR, Hassani Tabatabaei SS.

Medical School, Islamic Azad University, Yazd Branch, Yazd, Iran.

E-mail: hrsgmed@yahoo.com

Introduction: W.H.O statistics show that of the 123 million successful pregnancies per year, 87 million are unintended, there are approximately four to five thousand unintended pregnancies every year. The aim of the present study was to study the factors involved in unintended pregnancies in women referring to the Sh. Kargar Hospital of Yazd.

Materials and Methods: This descriptive cross-sectional study included 320 pregnant women referring to the Sh. Kargar Hospital of Yazd in the summer and spring 2006. A specially formatted questionnaire was used to collect their demographic characteristics and data about the contraceptive methods used and reasons for their failure. SPSS software program was used for analysis and statistical tests included Chi-square and ANOVA tests with a confidence interval of about 95%.

Results: The prevalent rate of unintended pregnancies in the population under study was 19.5%. The mean age was 26.3 ± 5.2 years. The mother's educational status and mean age had a significant relationship with unintended pregnancies. The mothers were mostly acquainted with oral contraceptive pills as a method of contraception and most of them used the withdrawal method for contraception.

Conclusion: It is proposed that women should be educated about the correct method of contraceptive use and this educational program should be regularly followed by the related organizations. Permanent methods of contraception like tubectomy and vasectomy are proposed for families who have enough children.

Key words: Unwanted pregnancy, Family planning, Yazd.

P-53

Effects of α -tocopherol and A23187 on normozoosperm motility and vitality

Fanaee H¹, Keshtgar S¹, Bahmanpour S², Kazeroni M³, Ghannadi AR³, Rostami S³.

1 Physiology Department, Shiraz University of Medical Sciences, Shiraz, Iran.

2 Anatomy Department, Shiraz University of Medical Sciences, Shiraz, Iran.

3 Shiraz Fertility and Infertility Center, Shiraz, Iran.

E-mail: keshtgars@yahoo.com

Introduction: The capacity of antioxidant production is low in sperms. An increasing in intracellular calcium induces acrosomal reaction (AR) and at the same time increases reactive oxygen species (ROS) production, which, in turn, enhances cell damage and cell death.

This study was designed to determine the effect of an antioxidant (α -tocopherol) on sperm motility and viability in control condition and in conditions which AR was induced by A23187 (a calcium ionophore).

Materials and Methods: Normozoospermic semen samples were obtained from 15 volunteers 20-30 years old after 3-5 days of sexual abstinence. Samples were washed, centrifuged and incubated in 37°C and 5% CO₂ until sperms swimm-up. Sperms were counted in the supernant and divided into five groups, each contained 2×10⁶ sperm/ml. Groups 1 to 5 were incubated for one hour with Ham's solution, 10 μ M A23187, 40 μ M α -tocopherol, 10 μ M A23187 + 40 μ M α -tocopherol, and ethanol, respectively in 37°C, 5% CO₂. Sperm motility assessed by grading system (A to D), which is recommended by WHO. Sperm vitality was determined by eosin staining.

Results: α -tocopherol did not induce any changes in sperm motility or viability, but a significant decrease in sperm rapid motility and viability was noticed after one hour incubation with A23187 as compared with the control group. On the other hand, incubation with α -tocopherol and A23187

(group 4) improved rapid motility and increased sperm viability compared to A23187 alone.

Conclusion: Treatment with α -tocopherol neither improves normal sperm motility, nor decrease cell death, but it can completely reverse the effects of A23187. It seems that, the adverse effects of A23187 are due to excessive ROS production caused by increased intracellular calcium.

Key words: Sperm motility, Vitality, α -tocopherol, A23187.

P-54

Association between air pollution (SO₂) and low birth weight

Araban M¹, Kariman N², Motesaddi S³, Alavimajd H⁴.

1 Nursing and Midwifery Faculty, Shahid Sadoughi University of Medical Science, Tehran, Iran.

2 Nursing and Midwifery Faculty, Shahid Beheshti University of Medical Science, Tehran, Iran.

3 Faculty of Health, Shahid Beheshti University of Medical Science, Tehran, Iran.

4 Paramedical Faculty, Shahid Beheshti University of Medical Science, Tehran, Iran.

E-mail: araban62@ssu.ac.ir

Introduction: Low birth weight as a result of premature labor or limited intrauterine growth is the most important measure of neonatal mortality. It has many causes of which air pollution has been recently gained attention. The Purpose of this historical cohort study was to determine the relationship between sulfur dioxide and low birth weight in term neonates of women referring to selected hospitals in Tehran in 2007.

Materials and Methods: 225 women referring to selected hospitals and residing at 5-Km distance from stations for air pollution measurement were selected. An information form was used to collect data. Its validity and reliability were achieved by content and test-retest methods respectively. Women were divided into 2 groups of exposure (low and high) according to their contact with the pollutant. The groups were matched in terms of factors effective on low birth weight.

Results: Findings showed a significant relationship between sulfur dioxide and low birth weight ($p < 0.5$, RR=3.96, CI=1.74-8.92).

Conclusion: With respect to the results, appropriate instructions should be provided for women residing at crowded areas of the city and necessary interventions carried out to decrease this pollutant.

Key words: Low Birth Weight, Sulfur dioxide (SO₂), Air pollution.

P-55

Unwanted pregnancy and related factors

Arabani M, Khoshbin A, Bokaei M, Karimian Z, Bahrami N.

Nursing and Midwifery Faculty, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

E-mail: arabani62@ssu.ac.ir

Introduction: Unwanted pregnancy is a worldwide problem and that has much health and socio economic problem. These problems consist of: Illegal abortion, infection, hemorrhage and post partum depression which they are directly related to mother and society health. Unwanted pregnancy is used as a health indicator. The Purpose of this cross sectional study was to determine the relationship between unwanted pregnancy and demographic features and socio-economic factors.

Materials and Methods: 225 women referring to Tehran selected hospitals in 2007 were selected. An information form was used to collect data. Its validity and reliability were achieved by content and test-retest methods respectively. Data collection was done by multi stage sampling. Data analysis was done by SPSS 15 and t test, χ^2 , factor analysis.

Results: Mean age was 25.86 ± 4.6 and mean gravity was 1/7. The prevalence of unwanted pregnancy was 28%. There was a significant association between unwanted pregnancy and maternal education, birth order, and socio-economic factors, $p < 0.05$. There was no significant association between unwanted pregnancy and maternal and paternal job, paternal education.

Conclusion: With regards to these results that shows a high prevalence of unwanted pregnancy especially in the first years after delivery and low socio – economic class women, correct and effective consultation with mothers about the necessity of the correct use of family planning methods and child spacing should be instructed by expert persons.

Key words: Unwanted pregnancy, Demographic features, Socio-economic factors.

P-56

The effectiveness comparison of two emergency contraception methods, Levonorgestrel versus Yuzpe

Farajkhoda T , Khoshbin A , Enjezab B, Bokaei M, Karimi Zarchi M.

Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

E-mail: farajkhoda_t@yahoo.com

Introduction: The spite of different contraception methods, unwanted pregnancy is a common problem in reproductive health. Unwanted pregnancy may lead to unsafe abortions. ECP is only method against unplanned pregnancy in our country, because of legal aspects. The prevalence of unwanted pregnancy is estimated 40 % in Iran; therefore it is essential that we select the best EC method. This study was designed in order to determine the effectiveness comparison of two emergency contraception methods, Levonorgestrel versus Yuzpe.

Materials and Methods: In a prospective, randomized, comparative study, we included 122 healthy volunteers (15 to 45 years women) who need to intake ECP in order to prevent unwanted pregnancy. The inclusion criteria at recruitment was occurrences unprotected sexual intercourse or condom accident. They randomly allocated in Levonorgestrel group (n=62 women) and Yuzpe (n=60 women) .The Levonorgestrel regimen consisted of two pills: 0.75 mg Levonorgestrel, taken twice in the 12-h interval within 72 hours after unprotected intercourse. The Yuzpe regimen included two HD contraceptive pills taken as Levonorgestrel method. Data were collected by questionnaire at first and three weeks later. The differences were compared with χ^2 and Fisher's exact tests.

Results: There were no statistically significant differences between the groups in number of gravity and parity, time of using the first dose of treatment. The Levonorgestrel regimen has been found superior to Yuzpe because it's more effectiveness and less side effects (respectively 100% vs. 91% $p=0.026$). With both PCC methods, no Ectopic Pregnancy occurred in all the women.

Conclusion: The study results illustrated more effectiveness and safety of the Levonorgestrel regimen as emergency contraception .It has been introduced as a gold standard of ECP and is used in USA, Europe and other countries. The study results suggested using of Levonorgestrel as an alternative ECP method instead of Yuzpe regimen as soon as possible in Iran.

Key words: Yuzpe, Levonorgestrel emergency contraception pills.

P-57

Thrombophilia tests in infertile patients with PCOS

Sarrafioun F¹, Ziaei S², Moeini A³, Faghihzadeh S⁴.

1 Tarbiat Modares University, Tehran, Iran.

2 Department of Obstetrics and Gynecology, Tarbiat Modares University, Tehran, Iran.

3 Department of Obstetrics and Gynecology, Tehran University of Medical sciences, Tehran, Iran.

4 Department of Biostatistics, Tarbiat Modares University, Tehran, Iran.

E-mail: f.sarrafioun@yahoo.com

Introduction: Pregnancy introduces a significant risk of Venous Thromboembolism (VTE) events. Many important changes in the coagulation and vascular system occur during pregnancy, so it is wrong to say that pregnancy is a state of hypercoagulation. Polycystic ovarian syndrome (PCOS) is also associated with insulin-induced elevations of plasminogen activator inhibitor-1 (PAI-1), the most potent inhibitor of fibrinolysis. In addition to hypofibrinolysis due to increased PAI-1 levels, miscarriage and complications in pregnancy are probably a result of prothrombotic effects of thrombophilia in PCOS patients. **Object:** To determine the value of thrombophilia tests in infertile patients with PCOS, and optimizing the treatment of these patients to protect them against thrombophilic and fetal complications.

Materials and Methods: This analytic case-controlled study was done in 2008 in Iran, on thrombophilia tests including Protein C, Protein S, Antithrombin III, APC-Resistance, and Homocystein levels for 125 infertile women with PCOS as the case group, and 76 infertile women with male factor as the control group. Both were matched for Age, BMI and Parity.

Results: The statistical results are summarized as the following:

Protein S deficiency test results: 23 patients (%18.4) in the case group, 10 patients (%13.2) in the control group, ($p=0.331$ no significant difference between the groups),

Antithrombin deficiency test results: 9 patients (%7.2) in the case group, 6 patients (%7.2) in the control group, ($p=0.843$ no significant difference between the groups),

and their medical history shows:

abortion rates: 32 patients (%25.6) in the case group, 9 patients (%11.8) in the control group, IUGR rates: 2 patients (%1.6) in the case group, no patients (%0) in the Control group, ($p=0.019$ a significant difference between the groups).

Conclusion: There is no significant difference in the thrombophilia factors between the infertile women with PCOS and infertile women with male factor. Abortion and IUGR rates in patients with

PCOS are significantly higher than normal women. There were only 201 patients in the study. It's possible to get more accurate results by having more cases and develop the study.

Key words: VTE, PCOS, Protein S, Apc-Resistance, Abortion.

P-58

A study of Araki gynecologist experts and educated midwifery's knowledge about ethical religious and legal roles of abortion article in 2006

Taheri A, Mobaseri Sh, Farahan H.

Arak University, Arak, Iran.

E-mail: Taheri.23359@yahoo.com

Introduction: Abortive embryo is one of the complex and complicated problems in today's societies. From ancient times, it has been as one of subjects of juridical and penal systems or organization. Abortive embryo in term of medical science is expressed as going out child before it could be alive. Abortive embryo is an unlawful or illegal form always has been along with damages such as severe or strong pain and bleeding, bacterial shock, DIC and acute renal failure and death. Illness and death jeopardize health's society and family and we should prevent its occurrence. In Islamic law any damage to child or mother that causes abortion is a crime and any one that commits the crime should be punished. According to Islamic punishment law, article of 622, any one abort an embryo intentionally, he or she should be sentenced to pay blood-money or to punish duly according to this case. Superficial looking to the documents of medical crimes shows that one of the causes of increasing amount of complaints from medical professionals was related to lack of knowledge of legal problems. Therefore, we decided to evaluate the rate or amount of knowledge of obstetricians and gynecologist in this field.

Materials and Methods: In a descriptive study, we asked people to fulfill a questionnaire including demographic and legal information about abortion. The data were analyzed by SPSS 11 software.

Results: The number of the sample was 75 people, %56 of them were 31–40 years old. %72 of them were obstetrician, %30.7 had a history of studying the academic field and %2.7 of them have read Islamic punishment law book. While %18.7 of them had no knowledge about this case.

Overall, %56 of individuals was opposed to abortion, while %36 of them agreed to abortion but

according to special circumstances. %56 of people, suggested appropriate methods of family controlling for decreasing illegal abortion.

Conclusion: The findings show that %80 of medical groups or midwives that are at the first line with this problem are opposed to abortion, unless in special circumstances. While only %8 agreed with abortion.

Key words: Gynecologist, Midwifery's knowledge, Enticed religious, Legal roles, Abortion.

P-59

Large volume leukapheresis allows achieving adequate yield of CD34 and MNC with single procedure in during of period of shorter time

Shahini F, Ali Moghadam K, Ostad A.

Hematology-Oncology and BMT Research Center, Shariati Hospital.

E-mail: dyarast.temi@gmail.com

Introduction: The peripheral blood stem cells (PBSC) have increasingly been used as a source in hematopoietic disorders and solid tumor. Despite this common use the best method and factors to achieve adequate Cd34⁺ cells and mononuclear cells (MNC) in the shortest time was not determined. The changeable parameters in software of the aphaeresis device play an important role in collection of stem and progenitor cell.

Materials and Methods: We enrolled in this study 80 healthy volunteer donors in two groups (40 donors in each group). All of them were mobilized with granulocyte colony stimulating factor at a dose of 5-10 mcg/kg/day during 4 days. Harvest started on day 5 using cell separator machines (Cobe Spectra version 7:00 and Fresenius AST-tec.204).

Results: In the first group a median of 14L of blood was proceed (12-24) with a median of inlet flow 70mL of blood during 160 to 280 minutes. Median of collected volume was 230mL. Proceed blood volume in the second group was selected between (8-12) with inlet flow of blood only 40 mL through leukapheresis of donor.

Conclusion: In two groups one aphaeresis procedure was required to obtain an adequate yield of stem and progenitor cells for transplantation (a median of 7×10^8 of MNC and a median 6×10^6 of CD34 cells). But we exclude the large volume leukapheresis during a shorter time to allow the collection of an adequate yield of peripheral blood stem and progenitor cells for transplantation for allergenic donation with single procedure.

Key words: Leukapheresis, CD34, Peripheral blood stem cell, Hematopoietic stem/progenitor cell transplantation, Instrumentation of apheresis.

P-60

Evaluation of endometrial morphology by scanning electron microscopy on the Clomiphene citrate comparison with Clomiphene citrate plus Estradiol Valerate and Clomiphene plus progesterone in infertile women

Favaedi M, Taheripanah R, Kabir Salmani M.

Infertility and Reproductive Health Research Center, Cell & Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

E-mail: taheripanahf@yahoo.com

Introduction: Induction of ovulation is one of the primary steps in the treatment of human infertility. It has been documented that administration of clomiphene citrate is the first step in the induction of ovulation.

Ovulatory rate with this Approach is about 80% but that for Pregnancy is about 40%. The aim of this research is the evaluation of the clomiphene effect on the endometrial morphology and pinopodes.

Materials and Methods: This study was a randomized clinical trial that was done from January 2007- March 2008 in Imam Hossein Hospital and infertility and reproductive Health research center in Iran. Study population was 30 infertile women who were clomiphene resistant with 100 mg/day clomiphene in the previous cycles. Patients were divided in 3 groups. In group 1, clomiphene 150 mg/day was administered from 5 -9 day of cycle, clomiphene plus Estradiol valerate to group II and clomiphene plus progesterone to group III. Endometrial Pinopods were compared in different groups on implantation window with Electron Microscopy. The progesterone, estradiol and endometrial thickness were measured in groups and compared together. Statistical analysis was based on kruskal- wallis method.

Results: The mean age of the patients was 26.81 ± 2.85 years. There were no significant differences in endometrial thickness on the day 14 between three groups of induction ovulation. There were no differences in groups for ovarian Follicles numbers and size and endometrial thickness in 14th day of Menstrual cycle, but in Clomiphene only treated group the serum progesterone was significantly higher compared to other groups in

21st day of cycle. Endometrial thickness was 7.55±1.74, 7.87±1.39 and 8.00±1.85 mm on the day of 14 cycles (p-value =0.903). Pinopodes were 27.72±21.8, 39.4±28.9 and 27.69±19.05, respectively. There were no meaningful differences between groups for pinopodes between three groups but the difference was significant when the Clomiphene- estrogen group was compared with other groups (p=0.05).

Conclusion: Adding estradiol valerate to clomiphene can be effective on endometrial morphology and increases the pinopodes on implantation window during induction ovulation. It seems anti-estrogenic effect of clomiphene reduce the endometrial pinopodes and decrease the rate of pregnancy and adjuvant therapy is effective.

Key words: Pinopodes, Clomiphene, Estrogen, Implantation window.

P-61

Diagnostic and operative hysteroscopy in women with infertility and abnormal uterine bleeding

Abdollahi-Fard S, Montazery F.

Department of Obstetrics and Gynecology, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: mo_oshaghi@yahoo.com

Introduction: Hysteroscopy as a modern instrument is available with CCD camera and cold light to inspection and operation of uterine cavity. Nowadays hysteroscopy as a minimally invasive

technique is used for diagnosis and treatment of common uterine disorders including: uterine polyp, myoma septum, bleeding and....

The aim of this study was evaluation of hysteroscopy application in infertility and abnormal uterine bleeding in Alzahra Hospital since 2006-2008 (2 years).

Materials and Methods: all patients with indication for hysteroscopy included and the results were analyzed; design of study was descriptive retrospective case note review including all admitted for hysteroscopy in two years.

Results: 110 cases of hysteroscopy was done for two years with mean ages 39.45±9.72, mean gravidity and abortion was 3±2.25 and 0.62±1.58 respectively. 40 (36.4%) patients had regular menses while 62 cases had irregular menses (56.4%). The most common indication was vaginal bleeding (96 patients) while in 12.7% the reason for admission was infertility or recurrent abortion. Diagnostic hysteroscopy was the only procedure in ten patients but other coincident procedures as curettage (38%), laparoscopy (12.7%), myomeresectomy (7.3%) and laparotomy (10.8%) were the most common operations with hysteroscopy.

Conclusion: Hysteroscopy as a minimally invasive method is the first choice for treatment of intrauterine disorders in patients with desire of fertility preservation with minimal complication.

Key words: hysteroscopy, vaginal bleeding, infertility.