K1 (Key Lecture 1): Hysteroscopy myomectomy

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Approximately 20% to 40% of women in reproductive age are known to have uterine myoma. It has been estimated that uterine myomas are associated with infertility in 5% to 10% of the cases. A number of mechanisms by which myomas cause reduced fertility have been suggested. According to hysterosalpingographic data, submucosal fibroids were classified as:
1. Submucosual fibroid with the greater position inside the uterine cavity.
2.Submucosal fibroid with larger portion located in the myometrium
3.Multiple (>2) submucosal fibroids.
Hystreroscopic myomectomy should be strongly considered in women with symptomatic intracavitary myomas particularly in view of the impact of intracavitary distortion in IVF patients. Myoma can be removed with hysteroscopy, scissors, electrocautery or laser treatment.

K 2(Key Lecture2): Laparoscopic myomectomy: should it be done?

Yao shu, zhong

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Uterine leiomyomas may cause menorrhagia, dysmenorrhea, and pelvic pressure. Certainly, it may have some relationship with fertility ability. The size of the myomas may enlarge as the women become old. It has the potential to have malignancy change if it exists for a long time. Therefore, myomas should be removed. Laparoscopic surgery has its benefit for the patients. It provides the advantages of shorter hospitalization; faster recovery, fewer adhesions and less blood loss than abdominal surgery for removing the myomas. As the improvement in surgical instruments and techniques, more and more cases of myomas can be removed under laparoscopy. Indication for surgical management of uterine myomas includes the followings: abnormal uttering bleeding, high level of suspicion of malignancy, infertility with distortion of the endometrial cavity or tubal occlusion, Pain or pressure that interferes with quality of life, Urinary tract frequency or obstruction, Iron deficiency anemia related to abnormal uterine bleeding. Preoperative Evaluation Women with leiomyoma present a history of pain, pressure, bowel or bladder symptoms and may have infertility or a history of pregnancy wastage. Couples with infertility should undergo a full evaluation to identify confounding factors such as male factor infertility or tubal occlusion before surgery. The pelvic examination may confirm uterine fibroids in some cases but transvaginal ultrasound evaluation must always be performed to evaluate the size, number and location of fibroids when laparoscopic myomectomy is considered. Hysteroscopy is appropriate if a submucus myoma or polyp is identified by transvaginal ultrasound evaluation. When the ultrasound findings or clinical presentation is atypical, magnetic resonance imaging (MRI) or computerized tomography (CT) scanning may provide enhanced diagnostic information and confirm the diagnosis of myomas or adenomyosis.

Laparoscopic Myomectomy Techniques

The goal of laparoscopic myomectomy should be a repair that is comparable, or superior, to closure of abdominal myomectomy. If comparable results are

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to be obtained with laparoscopic myomectomy, a three-layer closure (or four-layer, if the endometrium is entered) should be performed.

Trocar placement is critical to allow myoma dissection and counterattraction during enucleation, facile needle passage, pick-up, and assistance during suturing. The laparoscope is placed through an umbilical incision in most cases (A). It may also placed 3-4 cm above the umbilicus (D) for very large myoma. A 5 mm trocar S placed in the right side slightly below the umbilicus(C). A 10 mm trocar is placed on the contralateral side in the left (B). (Fig 1). Initially, a 5-mm reducer is placed over the 10-mm port for the use of 5 mm instrument. Eventually, the large port in the left will be replaced by the morcellator trocar to remove the specimen.

A manipulator probe is placed into the cervix to move the uterus into optimal position during enucleation and suturing. The manipulator probe should not be used for a virgin with laparoscopic myomectomy. Once the instruments are placed, a thorough assessment of the pelvis is performed, and the feasibility of performing laparoscopic myomectomy is reassessed. Conversion to laparotomy in difficult cases should be considered a sign of wisdom, not evidence of defeat. Occasionally, a deep intramural myoma with small size cannot be localized by laparoscopy. When needed, intra-operative transvaginal ultrasound can help direct the surgeon to identify the location of the myoma, and identify the optimal site for the uterine incision. The uterus is injected with undiluted pitressin (12 units). This drug can cause strong contraction of the uterus and helps to reduce blood loss, decreases surgical time, and increases the ease of resection .We inject 12 unit pitressin into the uterus at one sites with the needle placed directly through the abdominal wall. After the injection of pitressin, a vertical elliptical incision or

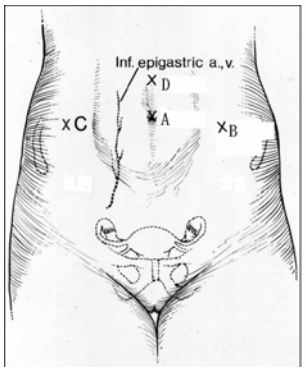


Fig 1: Port placement site

linear incision were made to exposes the myoma. Once the myoma is exposed, a 10 mm claw forceps was placed through the 10 mm trocar in the left to grasp the myoma for counter traction against the myometrium, to identify tissue planes, and rinse the surgical site. A 5 mm hook scissor were placed through the 5 mm port in the right to dissect the myoma from the myometrium .The scissor may facilitate cutting the pseudocapsule and enucleate the myoma. Keeping the scissor in direct contact with the myoma reduces the chance of entering the endometrial cavity. The myomectomy dissection by laparoscopy is usually remarkably clean and relatively bloodless. Once removed, the myoma is placed in the anterior or posterior cul-de-sac. It can also be placed in the right iliac fossa for large myoma. Both transverse and vertical incision can be used for enucleation of myoma. The selection of incision direction is depending on the needle driver placement. Both vertical and transverse incision in the uterus can be sutured with ease. Closing the incision is technically the most challenging aspect of laparoscopic myomectomy, but advances in surgical techniques allow for excellent approximation of all layers. Here, I emphasize the suture technique which is very important in laparoscopic myomectomy. You can

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not finish the procedure if you can not closing the incision using endo-suture technique. The needle driver is held in the surgeon's right hand, and a grasper forceps is held in the surgeon's left hand. The incision was sewed. In other words, passing the needle into and out of the tissue layers with the needle driver in the right hand, then grasps the needle with grasp forceps in the left hand. We don't close the endometrium even if the endometrium is entered during the myomectomy. Usually we close the deep layer of myometrium with 1-0 vicryl first and than close the myometrium layer by layer using 1-0 or 1 vicryl. We prefer a continuous running suture and intracorporeal ties, as tension on the knot and visualization is good with intracorporeal tying .The fibroids are removed with a mechanical morcellator. The 10-mm trocar sheath is removed from the left side, and replaced with the morcellator trocar. The myomas was grasped with the 10 mm claw forceps and pulled into the activated morcellator. Myoma strips and fragments are removed through the morcellator channel. Extreme care must be taken to avoid injury to abdominal organs during morcellation, and the cutting blade is retracted except when morcellating the myomas. After removal of myomas, the uterus is irrigated and homeostasis confirmed. The myomectomy incisions are covered by anti adhesion materials such as Interceed to reduce adhesions after homeostasis has been achieved. The 10mm and morcellator trocars increase the risk of herniation into the internal facial sheath, and this layer should be closed separately to avoid herniation. Postoperative recovery after laparoscopic myomectomy is longer than diagnostic laparoscopy, but most women may return to full activities within 1–2 weeks of surgery. Active ambulation is advised. Regular diet is initiated as tolerated, usually after the postoperative nausea from anesthesia is resolved. Laparoscopic myomectomy provides an acceptable, and perhaps a preferable, alternative to abdominal myomectomy for women with symptomatic fibroids who desire uterine preservation, and appears to be an excellent alternative to abdominal myomectomy for women who have infertility primarily related to fibroids. Laparoscopic myomectomy clearly provides a more rapid recovery compared to abdominal myomectomy, and is usually associated with less blood loss and fewer adhesions compared to an open approach. Pregnancy rates are comparable to those expected with abdominal myomectomy and the risk of uterine rupture during pregnancy is less than 1% if the uterus is closed appropriately. Advances in surgical instruments and techniques have expanded the role of laparoscopic myomectomy in well selected individuals. Meticulous repair of the myometrium using microsurgical principles is essential for women considering pregnancy after laparoscopic myomectomy to minimize the risk of uterine rupture. Although laparoscopic myomectomy have many benefit for the patient, it can not be used for women with multiple fibroids, especially for those with multiple intramural myomas. The reason is that we can not find small intramural myomas without finger palpation as we did during abdominal myomectomy. It may cause the residue of the fibroids. Therefore, I emphasize that we can perform laparoscopic myomectomy but in well selected cases.

K3 (Key Lecture 3):
Maternal communication with gametes and embryos

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The majority of adult chronic diseases such as coronary heart disease, hypertension, stroke, type 2 diabetes, obesity, osteoporosis and cognitive impairment have been shown in numerous epidemiological studies from diverse world populations to have *in utero* origins, associated with poor maternal nutrition and physiology ('Foetal Origins' Hypothesis). Using different animal models, the initiation of altered developmental potential associated with postnatal disease can be traced back to the periconceptional period and the cross-talk between the maternal reproductive tract and the preimplantation embryo. Thus, several studies have shown that cleavage stage embryos are sensitive to environmental conditions which can permanently alter the developmental program leading to abnormal postnatal growth, metabolism, physiology and behavior. Identifying the environmental factors that can influence embryonic developmental potential through to adulthood would have major public health implications, and provide a basis for the prevention of common diseases. The mechanisms by which these early life exposures may mediate such profound long-lasting consequences are poorly understood. At this stage, we are certain that presence of gametes and embryo in the female reproductive tract influence the reproductive tract environment and consequently alters the micro-environment of gametes and embryos. But how mothers can regulate changes in maternal environment in response to gametes and embryos is not known. This is a complex issue of integrated physiological dimensions which requires complete characterization and understanding of maternal cross talk with gametes and embryo. During the presentation I would try to highlight some advances related to characterization of maternal cross-talk with gametes and embryos and the effect that this cross talk may have on further embryonic development and health in adulthood.

K4 (Key Lecture 4): Endometrial nerve fibres in endometriosis

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There is increasing evidence to indicate that endometriosis is an endometrial disease and is greatly influenced by genetic predisposition. We have started to explore ways in which the endometrium could be involved in the mechanisms of generation of pelvic pain in endometriosis.Women with pelvic pain, infertility or no uterine symptoms, who have undergone laparoscopy or hysterectomy with confirmation or exclusion of endometriosis or other pelvic pathology. None were on hormonal therapies. All have had endometrial tissue sampling. Over 100 endometrial tissue samples have now been studied.Endometrial samples were fixed in 10% buffered formalin, blocked in paraffin, sectioned at 4 microns and stained in an auto-stainer with conventional H&E, or with a series of immunohistochemical markers (pan-neuronal marker, PGP9.5; vaso-intestinal peptide, VIP; neuropeptide Y, NPY; neurofilament, NF; substance P, SP; calcitonin gene-related peptide, CGRP; tyrosine hydroxylase, TH; acetyl choline, ACh; nerve growth factor, NGF; nerve growth factor receptor, NGFR).All endometrial samples from women with confirmed endometriosis have been demonstrated to have fine, unmyelinated, sensory nerve fibres in

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the functional layer. These predominantly expressed VIUP and NPY, with smaller numbers expressing CGRP and SP. Women without endometriosis were never found to have fine nerve fibres in the functional layer. Endometriosis sufferers had a higher density of nerve fibres of different types throughout the basal layer and the myometrium than women without endometriosis. NGF and NGFR were also expressed to a much greater degree in the functional layer of eutopic endometrium of women with endometriosis. Fine sensory nerve fibres have also been demonstrated in all plaques of peritoneal endometriosis.Fine sensory nerve fibres are present in the functional layer of eutopic endometrium in all women with endometriosis. these are not present in women without endometriosis. This finding is so consistent that endometrial biopsy may be used as a reliable means of making the diagnosis of endometriosis. These nerve fibres may play a role in the generation of endometriosis pain, both within the uterus (endometrium and myometrium) and in ectopic endometriotic plaques.

K5 (Key Lecture 5):
Failed fertilization post ICSI and spermiogenic Defects

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To evaluate the relationship between some events that simultaneously occur during spermatogenesis, related to protamine deficiency, acrosome integrity and sperm morphology with fertilization rate post ICSI, Semen samples from 68 infertile couples undergoing ICSI at Isfahan Fertility and Infertility center were assessed during this study. Semen analysis was carried out according to WHO criteria. Protamine deficiency, acrosin activity, sperm morphology and acrosome size were assessed by Chromomycin A3 (CMA3) staining, gelatinolysis test and Papanicolaou staining (Strict criteria), respectively. Couples were sub-grouped according to CMA3 positivity of lesser and greater 40% criteria. Percentage CMA3 positivity and mean halo diameter show a significant correlation with fertilization rate. However, no correlation was found between sperm normal morphology and fertilization rate. The mean values of acrosome size and fertilization rate were significantly different when patients were grouped for CMA3 positivity of 40%. Multiple linear regression analysis reveals that only protamine deficiency has direct effect on fertilizion rate ($r = -0.5$, $P < 0.001$).Protamine deficiency appears to have a more significant effect on fertilization post ICSI compared to acrosin activity and semen parameters.

K6 (Key Lecture 6):
Identification of mouse embryonic stem cell-associated proteins: a comparative proteomic approach

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Over the past few years, there has been a growing interest in discovering the molecular mechanisms controlling embryonic stem cells (ESCs) proliferation and differentiation. Proteomic analysis showed to be an effective approach to comprehensively unravel the regulatory network of differentiation. We applied a two dimensional electrophoresis-based proteomic approach followed by mass spectrometry to analyze the proteome of two mouse ESC lines, Royan B1 and D3, at 0, 6 and 16 days after

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differentiation initiation. Out of 394 differentiation associated proteins detected in two lines, 97 were common. Of them, 51 spots were down-regulated and 17 spots were up-regulated in differentiated cells compared to ESCs in both lines and six protein spots observed only at proliferating ESCs. Mass spectrometry analysis of these protein spots led to identification of 81 proteins. The expression pattern of four down-regulated proteins including Hspd1, Hspa8, β-actin and Tpt1 were further confirmed by western blot and immunofluorescence analyses in Royan B1 and D3 as well as two other mouse ESC lines, Royan C1 and Royan C4. Differential mRNA expression analysis of 20 genes using quantitative real-time reverse transcription PCR revealed a low correlation between mRNA and proteins levels during differentiation. We also observed that the mRNA level of Tpt1 increased significantly in differentiating cells whereas its protein level decreased. Several novel ESC-associated proteins have been presented in this study which warrants further investigation with respect to the etiology of stemness.

K7 (Key Lecture 7): Making germ cells from stem cells

Nayernia, K; Lee, J.H; Nolte, J; Michelmann, H.W; Rathsack, K; Drusenheimer, N; Dev, A; Wulf, G; Ehrmann, I.E; Elliott, D.J; Okpanyi, V; Zechner, U; Haaf, T; Meinhardt, A Engel, W.

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Germline stem cells (GSCs), which can self-renew and generate differentiated progeny, are unique stem cells in that they are solely dedicated to reproduction and transmit genetic information from generation to generation. We developed a strategy for the establishment of germline stem cell lines from embryonic stem cells (ES). These cells are able to undergo meiosis, generate haploid male gametes *in vitro* and are functional, as shown by fertilization after intra-cytoplasmic injection into mouse oocytes. Molecular and cellular mechanisms underlying differentiation of ES to functional gametes should be elucidated in future research. In other approach, we show that bone marrow stem (BMS) cells are able to Trans-differentiate into male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells. The ability to derive male germ cells from ES and BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine

K8 (Key Lecture 8): Pluripotent stem cell invasion and the role of organ stroma

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Pluripotent stem cell transplantation is one of the most exciting areas in biomedical research today. Since, it holds the promise of being a central instrument in clinical regenerative medicine. However, the development of long-term successful regenerative therapies critically depends on the deepening of our understanding of the cellular and molecular bases of the maintenance of proliferation and pluripotency, which also will contribute to a better understanding of embryonic development and cancer progression. Recently, it has been reported that stem cell treatment, in addition to providing symptom relief for Parkinson's disease, can also have cancerous side effects. Even more surprising is the finding that

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mammary stroma can redirect the cell fate of transplanted spermatogonia *in vivo*. Overall, these findings suggest that it is not only the nature of the pluripotent cell which is vital, but also the characteristics of the microenvironment into which the cell is transplanted. We understand that it is thus important to further characterize the crucial interactions of pluripotent stem cells with organ stroma, as well as the invasive mechanisms employed by these cells. In my talk, I am honored to present to you the experimental approaches we have employed to examine stroma-cell interactions by studying the invasive activity of a group of functionally related pluripotent stem cells: ES cells, EG cells (from PGCs) and EC cells.

K9 (Key Lecture 9): Laparoscopic microsurgery of fallopian tubes

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Tubal factor infertility is one of the most common causes of infertility and there are two ways for treatment of this problem. One is *in vitro* fertilization and the other is tubal surgery. Tubal surgery should be performed by microsurgery technique to have a better result. In selected cases microsurgical tuboplasty has a better result in comparison to IVF. Traditionally microsurgery was performed by open laparotomy. Thus there are a few centers in the world that do this procedure by laparoscopic. In this lecture, benefits and disadvantages of laparoscopic microsurgical tuboplastic will be discussed.

K10 (Key Lecture 10): The possibility of developing trophoblast cells in vitro

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Trophoblast differentiation and early stages of placental development are important events for the establishment of pregnancy but due to ethical and practical problems these critical events are not readily investigated in human pregnancy. Human embryonic stem cells and their malignant counterparts, embryonal carcinoma cells and the cells from the inner cell mass of human blastocysts closely resemble one another and can be used as an *in vitro* model for investigating the properties of trophoblast cells and early human development. Different cell lines have been generated from normal, malignant or transformed trophoblast and placental tissues. Since, many of these cell lines originate from late or term placental tissues or have lost their function after transformation, they cannot recapitulate the physiology of trophoblast during the peri-implantation stages of development. Here, we have investigated the possibility of generating trophoblast cells from both human embryonal carcinoma and human embryonic stem cells and also generated human cytotrophoblast stem cell lines as a source of progenitor trophoblast cells to serve in model systems of implantation.

K11 (Key Lecture 11): Bioethical issues of the new reproductive technologies

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Reproductive Biomedicine and assisted reproductive technologies (ART)

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belong to the fields of medicine that initiated most of the discussion on enhancement and desire fulfilling medicine in bioethics. In the past few decades, new and more efficient techniques have become widely available to solve fertility problems. It is important to learn about different socio-cultural and religious perspectives related to reproductive and genetic health problems. Religious groups are active in influencing the public regarding bioethical positions, and this is particularly evident with issues concerning procreation, abortion and infertility therapy. A questionnaire survey was administered to a random sample of population and infertile couples regarding their attitudes towards genetic testing and interest in such testing and opinions about donation. The results showed that their knowledge was low but the majority urged to become familiar with above testing and everything should be confidential. Infertility treatment is affecting by the diverse background, society, culture and religion. Any debate on the social, legal and ethical issues surrounding ART must be reminded to infertile couples. The information should be explained to them before they decide to go for the treatment. Understanding ethical issues even in genetic testing should be components of the population education. These educations should be considered as the first to protect the privacy of individuals, while considering genetic information needs of family members. Determination of appropriate monitoring of genetic tests is very important and current policies recommend that every one should be tested if evidence indicates that testing is in the best interests of them.

K12 (Key Lecture 12):
The promises of stem cells in reproductive medicine

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Nowadays, stem cells are subjected to immense research interest, and in the near future, are expected to provide the basis for a number of new treatment approaches for many life-threatening and severe disorders. Several stem cell types and sources have already been identified and characterized according to the tissues/organs that they are originated. Although embryonic stem cells, placental and cord blood stem cells are within the boundary of reproductive medicine, there exist relatively few studies on the potential use of these cells for research and therapy in this field. On the other hand, the potential applications of stem cells in the field of reproductive medicine are in fact outstanding: From the diagnosis perspective, the research that can be performed with embryonic and fetal stem cells can lead us to a better understanding of the cellular and molecular mechanisms of events such as infertility, genetics aspects of human gamete and embryo development as well as implantation. Emerging evidence also suggests that, besides their potential for basic research, stem cells may also have potential in the treatment of certain reproductive problems. So far, stem cells have been identified in diverse tissues of an adult, including human bone marrow, breast, prostate, brain and liver. Due to its cyclically regenerating nature, it has been hypothesized that endometrium also contains a highly proliferative epithelial and stromal cells that exhibit stem/progenitor cell behavior in vitro. The progeny in these colonies have been characterized and growth factors supporting clonogenicity are partially identified. Recent research now aims to reveal the potential of these cells in the

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diagnosis as well as in the treatment of endometrium-related disorders. Stem cells that are obtained from adult ovaries or testis have also created new research and treatment options for infertility. They not only become key tools for understanding the basic mechanism of gamete differentiation, but also help us to define novel protocols for in vitro production of gamete cells. Several recent studies have already provided scientific evidence that it can now be possible to produce gamete cells from embryonic stem cells by genetic modification and directed differentiation. However, more studies are required to provide and confirm the potential use of this new information in reproductive medicine. This presentation primarily focuses on the biological aspects and treatment potentials of different stem cell sources from reproductive medicine perspective. It further discusses the recent advances and results of current research projects in this field.

K13 (Key Lecture 13): Reactive oxygen species and infertility

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Reactive Oxygen Species (ROS) are a group of free radicals that are produced by spermatozoa, like other living cells in male reproductive tract. Under physiological conditions, spermatozoa produce small amounts of ROS. In minimal amounts, they are needed for regulation of sperm function, sperm capacitation and acrosome reaction; however at high concentration they are toxic to normal cells. Spermatozoal membrane is rich of polyunsaturated fatty acids and so is susceptible to lipid peroxidation when exposed to high amounts of ROS. Other studies showed that the amounts of ROS produced by spermatozoa were negatively correlated with the quality of sperm in the original semen. High ROS levels can also affect sperm fertility potential by DNA damage and increased apoptosis. A few studies reported that the levels of antioxidants in seminal plasma of fertile men are significantly lower than its levels in infertile controls. Also it is reported that up to 40% of infertile men may have high seminal ROS level. Increased production of ROS can also cause a decrease in axonemal protein phosphorilation and reduce sperm motility. High levels of ROS have also been correlated with poor semen morphology. We conducted two studies in our center respecting the role of ROS and infertility in our patients. First study showed that level of ROS in seminal fluid of infertile men is significantly higher than fertile donors and also in infertile patients with varicocele is higher than patients with unknown cause. The second study on infertile men with varicocele showed that varicocele is an important factor in producing ROS in seminal fluid and this effect increase with severity of varicocele. Recent studies showing that ROS production can be also an important factor in female infertility and the success of ART procedures.

K14 (Key Lecture 14): Myoma and reproduction

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Fibroids are the most common benign uterine tumors. A relation between fibroids and infertility is suggested. Successful implantation depends on a normal, receptive endometrium. Fibroids can change the normal appearance of the uterine cavity and can adversely affect the endometrium. It

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seems that treatment of fibroids in infertility patients is useful but this is not yet proven scientifically. The incidence of fibroids is associated with infertility in 5% to 10%. When all other causes of infertility are excluded, fibroids are responsible for only 2% to 3% of infertility cases. The mechanism of fibroids is interference with sperm or ovum transport, enlargement and deformity of the uterine cavity, uterine contractility, distortion of the cervix, distortion of obstruction of the tubal ostia. The management of fibroids is expectant, medical and surgery managements.

K15 (Key Lecture 15):
Introduction of newly designed instruments for laparoscopic operations

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Laparoscopic surgery requires heavy reliance on complex equipment. Several factors should be considered at the time of choosing laparoscopic instrument, including cost, availability and reliability. The operative outcomes depend on skill of surgeon and availability of Instruments. Some newly designed instrument for laparoscopic ovarian cauterization, myomectomy & laparoscopic hysterectomy (LH) will be presented. Reusable instruments are expensive initially but in long rum they are cost effective. The disposable instrument cost is less compared to re-usable but patient cost is increased. The main advantage of disposable instrument is high performance due to its sharpness and reduced chance of disease transmission due to certified high-end factory sterilization. In many centers re-use of disposable instrument is seen. In developing countries, disposable instruments are very rarely used because labour cost is low compare to the cost of disposable instrument. In Europe and USA, surgeons often choose to use disposable instrument. However, once discarded, environment concerns are raised about disposal and biodegradability of disposable instruments. Ideally, disposable instrument should not be used repeatedly because handling, sorting, storing and sterilization make these instruments questionable. The disposable instruments are not sterilized properly by dipping in gluteraldehyde because they are not dismountable. Insulation of disposable instrument also can be torn easily which can lead to electrosurgical injuries.

K16 (Key Lecture 16):
Simultaneous fetal cell detection and genetic diagnosis by immunophenotyping and chromosomal fluorescence in situ hybridization

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The isolation of fetal cells from maternal blood is presently in progress in many laboratories worldwide and several procedures have been described, although a routine clinical test is not yet available .Whichever procedure will eventually be the most successful, the limited number of fetal cells available for genetic analysis will represent a technical challenge or a limiting factor for routine investigation. Interphase cytogenetic by FISH is the standard approach in genetic investigation of fetal cells isolated from maternal blood. This approach has two major limitations, first target cells are distributed between a background of a large number of maternal cells, FISH

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analysis therefore results in a tedious and error-prone procedure in which fluorescent spot-like signals have to be scored in many hundred of thousands of cells while constantly changing the plane of focus in order not to miss signals from out-of-focus planes. Recovered cell population is therefore never analyzed in its entirety. An automatic FISH scoring system would represent a major improvement. Secondly an unresolved difficulty with this approach is the inability to distinguish a fetal cell unequivocally before the analysis of its chromosome constitution. Both these inconveniences would be solved by combining FISH evaluation with fetal cell in situ identification through a specific fetal-cell marker. The search for an antibody that recognizes an antigen that is unique to fetal cells has been so far unsuccessful. Combined immunocytochemistry (ICC) with Fluorescence in situ hybridization (FISH) has been described so far only with anti-ε and anti-γ globin chains monoclonal antibodies which recognize only a limited percentage of fetal cells, they entirely miss fetal CD34 stem cells. An interesting alternative might be a monoclonal antibody anti-i. This antigen predominates on fetal cells early in pregnancy and is very rare in maternal cells. In this report we describe several different procedures for simultaneous detection of i-antigen, and sex chromosome identification. The present study represents the necessary preliminary step to develop a suitable protocol to the applicability of automated microscopy and image analysis for the simultaneous detection of fetal cells and genetic diagnosis by FISH.

K17 (Key Lecture 17):

Role of laparoscopy in the management of cervical cancer

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When compared with laparotomy, laparoscopy provides a similar outcome with a shorter hospitalization, an earlier recovery, and an improved quality of life for the treatment of cervical cancer. We and others have demonstrated the feasibility and outcome of laparoscopic radical hysterectomy with or without pelvic lymphadenectomy for patients with early stage cervical cancer. Recent reports in the literature on cervical cancer management now include follow-up data; the 2-year disease-free and overall survivals were similar in patients treated by laparoscopy and laparotomy in a study that included a control group. One of the challenges for the development of laparoscopic surgery is the difficulty for physicians to acquire advanced laparoscopic surgical skills. The feasibility and safety of laparoscopy in the management of cervical cancer is now established from cohort or case–control analytical studies. The absence of large phase III studies needs to be balanced by the relatively low incidence of cervical cancer in the developed countries.

K18 (Key Lecture 18):
High survival- and pregnancy rate of vitrified and rewarmed human zygotes in contrast to the slow-cooling cryopreservation method

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Slow-cooling (SC) cryopreservation of supernumary pronuclear stage oocytes during IVF/ICSI is well established and routinely implemented in the clinical IVF-programme in Germany. Recently, worldwide excellent results for Cryo-Embryotransfer (Cryo-ET) by vitrification (V) (ultra-rapid cooling) using minimum volume cooling method have been reported. The radical strategy of vitrification results in total elimination of ice crystal formation, both within the cells being vitrified (intracellular) and the surrounding solution (extracellular). In the present study, we examined the survival rate of vitrified and rewarmed human zygotes that were cultured for additional 24 h before Cryo-ET and evaluated the pregnancy rate. The results were compared to survival- and pregnancy rate using the slow-cooling cryopreservation method. Between January 2000 and November 2005 in total 752 patients had 3616 supernumary zygotes during IVF/ICSI treatment. These zygotes were cryopreserved using the slow-cooling method (see: Al-Hasani et al., Hum Report 1987, pp 695-700). A total of 332 supernumary zygotes from 66 other patients were vitrified between March and November 2005 using the Cryotop (Kuwayama, RBM-online, 2005, pp 608-615). For vitrification, zygotes were placed into equilibration solution (7, 5% Ethylenglycol; 7, 5% DMSO) and incubated for 5-10 min. at room temperature (RT). Hereafter zygotes were incubated in vitrification solution (15% Ethylenglycol; 15% DMSO; 0,5M Saccharose) for 45-60 sec. at RT and placed on the Cryotop-strip and were plunged directly into the liquid nitrogen. After Vitrification, a hard plastic cover is attached to protect the strip during storage in liquid nitrogen. In total 1438 zygotes were thawed according to the conventional SC-protocol. 107 zygotes were rewarmed after being vitrified: the hard plastic cover was removed in liquid nitrogen and the Cryotop is plunged in thawing solution (1M Saccharose) at RT for 1 min. Zygotes are placed in diluent solution (0.5 M Saccharose) at RT for 3 min. and for another 5 min. at RT in washing solution (culture medium). After both procedures, vitality of zygotes was evaluated before they were cultured for additional 24 h to reach the stage of an embryo and was replaced into the uterus. Clinical pregnancies per Cryo-ET were evaluated and compared for both methods. In total 1438 zygotes were thawed after being cryopreserved with the SC method. 848 zygotes showed vitality after thawing, being equivalent to a survival rate of 59%. 107 zygotes were rewarmed after being vitrified corresponding to a survival rate of 77%. An increase of 18% percentage-points could be demonstrated, favouring the vitrification protocol. 583 patients underwent Cryo-ET after SC of zygotes. The clinical pregnancy rate per Cryo-ET was 19% (n=111). In contrast 32 patients underwent Cryo-ET after vitrification of zygotes. Pregnancy rate was 46.8% (n=15). Out of these 2 healthy babies were already delivered. This signifies an increase of 27,8 percentage-points by using vitrification. These first German results clearly demonstrate, that the Cryotop vitrification method of supernumary zygotes results in high post-thaw survival and pregnancy rates suggesting that the Vitrification-protocol may be a preferable, cost-effective, simple clinical cryopreservation method for human zygotes, oocytes and embryos in IVF/ICSI-cycles.

K19 (Key Lecture 19):
Management of hypogonadotropic hypogonadism in male patients

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Hypogonadotropic Hypogonadism (HH) is the etiology of infertility in about 1% of infertile males. HH is classified into congenital and acquired. In acquired HH prognosis works better and the result of management is better than that of congenital form.The chief complaints differ from age to age. At about puberty, patients refer with hypoandrogenism symptoms, but at later ages, the complaint is about infertility. The management of HH patients with these two presentations includes androgen supplementation and clomiphene, Gonadotropins, as well as GnRH, respectively. There are two interesting points about the management of these patients: first, The success of medical therapy in azoospermia and second, the occurrence of pregnancy in the female partner . In spite of the male partners oligospermia. In the first place, HCG is prescribed but in case that spermatogenesis does not occur, HMG is added. GnRH pump can be also used. Although it has no advantage over HCG+HMG.The present review aims at the time and dosage of the above-mentioned drugs, as well as the side effects, the success rate and the alternative managements.

K20 (Key Lecture 20): Expression and physiology of kinin receptors in the male reproductive tract

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Kinins are peptide hormones mediating their biological effects via specific receptors. The setting of a local tissue kallikrein kinin system (tKKS) within the reproductive organs of the male rat was investigated by analyzing bradykinin subtype 2 receptor (B2R) gene expression and cellular distribution of B2R protein and the kinin-liberating protease tissue kallikrein (tK). B2R gene expression was observed in testis, epididymis and prostate from prepubertal and sexually mature rats. In mature testis, B2R mRNA and protein are localized besides endothelial and muscle cells of blood vessels exclusively on pachytene spermatocytes and round and elongated spermatids. B2R expression within the seminiferous tubules (SFT) was dependent on the stage of the spermatogenic cycle. In prepubertal rats, the kinin receptor was additionally located in peritubular cells (PTC) surrounding the SFT. In the testis, specific staining for tK occurred on blood vessels and on the acrosomal cap of round and elongated spermatids. Here, this immunostaining was also stage-dependent. In the epididymis, tK was detected on epithelial cells near the apical surface. To further investigate the physiological function of the tKKS, we investigated the pharmacological properties of B_2R and relevant signal transduction pathways in PTC. Saturation and displacement experiments were performed using [^{3}H] bradykinin. Scatchard analysis of the binding data displayed a dissociation constant (K_d) of 1.13 nM and a maximal receptor density (B_{max}) of 22.34 fmol/mg Protein. The IC_{50} values were 7.9 nM for the B_2R-specific antagonist HOE 140 and 302 nM for the B_1R-specific antagonist [desArg] HOE 140. To examine which B_2R signal transduction pathways are involved, peritubular cells were stimulated with bradykinin or controls. The 2nd messengers IP_3, Ca^{2+}, cAMP and cGMP were measured using RIA, FURA-2 imaging or ELISA. Protein

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phosphorylation was determined using western blots. Bradykinin induced an rapid and significant increase in intracellular [Ca] $^{2+}$ via the B_2R, followed by a temporary rise in the IP_3 level. No changes in cAMP or cGMP levels could be detected. Bradykinin also induced a time-dependent phosphorylation of tyrosin residues of several proteins. The age- and stage-dependent specific expression of tK and kinin receptor within the SFT and epididymis and the pharmacological data suggest a potential role of the tKKS in the local regulation of spermatogenesis and sperm maturation but also SFT function.

K21 (Key Lecture 21): Status of prenatal diagnosis of genetic disorders in Iran

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Genetics disorders pose a major health burden for most families and societies. In Iran, particularly, this is even a problem since a major campaign against infectious diseases has been most successful and with the improvements of health and wealth of Iranians more attentions are paid to non-communicable disorders. Genetics disorders play important part on the list. The Iranian government has put lots of emphasis and attention of the prevention of genetics disorders in the past 10-15 years. With the implementation of National Program for the Prevention of Thalassemia (and other hemoglobinopathies) in 1997, this program received an important boost. This program is now more than 10 years old and we have learned a lot through this program. We have been able to receive religious decree or FATWA for selective abortion. Selective abortion has enjoyed a ruling passed in the Parliament. We have been able to obtain the insurance companies for covering the costs of doing prenatal diagnosis (PND). We have created a national PND Laboratories Network and National Reference Laboratories for PND. We have been able to reduce the birth of affected thalassemic children to unbelievable low number of less than 200 in year 2006 from expected 3000. We have recently started a similar and yet a more comprehensive program on PKU and hemophiliac. More genetic disorders are on the pipeline. It is needless to say that the Genetics Office at the Center for Disease Control, Ministry of Health is master mining the program. A similar initiative has been initiated by the Welfare Organization on the National Genetic Counseling Program. Through this program most parts of the country now have at least a genetics counseling unit. We think through these program, we will notice progress on the recognition of new cases, a more effective prevention program, a better management of affected children, more and better research projects on the genetic disorders and overall a more comprehensive growth of molecular medicine in Iran.

K22 (Key Lecture 22): Cochlear repair after transplantation of human cord blood cd 133+ cells to Nod-scid mice made deaf with kanamycin and noise

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We investigated whether human cord blood $CD133^+$ hematopoietic stem cells (HSC) engraft the cochlea contributing to its repair, *in vivo,* in *nod-scid* mice that had been made deaf after treatment

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with kanamycin and/or intense noise. PCR analysis, using HLA.DQα1 as a marker for the traceability of the engrafted cells, identified human HSC migrated to various organs of the host, particularly liver, spleen and cochlea, providing evidence of engraftment in injured tissues. All the DNA isolated from mice that did not receive a transplant remained negative. Histologic and immunofluorescent staining confirmed that HSC contributed to the *ex novo* phenotypic recovery of the internal apparatus of the inner ear, stimulating a rapid regeneration of hair cells in the organ of Corti (OC), a different situation to that of the control oto-injured mice that were not transplanted, that remained with seriously damaged or destroyed cochlear tissues. Dual color-FISH analysis detected the presence of both human and mouse centromeres both in separate cells and within a single nucleus. Most chimeric engrafted cells were of entirely human origin. Confocal scan analysis showed also a small number of heterokaryons, possibly derived from fusion of donor with endogenous cells. Within the cochlea, chimeric cells were seen predominantly in zones with abundant mesenchymal cells and fibrocytes, never in hair cells and neurons. These findings demonstrated that trasplanted human HSC migrating to the inner ear of oto-injured mice, contributed, *in vivo*, to generate new conditions for the phenotypic resumption of the cochlear OC neurons and hair cell loss.

K23 (Key Lecture 23): The beneficial aspects of *in vitro* oocyte maturation (IVM) in PCOS patients: Is it a sufficient procedure?

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Patients with polycystic ovarian syndrome (PCOS) are characterized by abnormal endocrine parameters, anovulation numerous antral follicles within their ovaries and frequently infertility. PCOS patients can be extremely sensitive to exogenous gonadotrophin and at risk of ovarian hyper stimulation syndrome when treated with gonadotrophins for assisted reproduction. In-vitro maturation (IVM) of immature oocytes from PCOS patients would be an attractive option to eliminate this problem and immature oocyte recovery could be developed as a new method for treatment of patients with infertility due to PCOS because the oocytes of these patients retain their maturational and developmental competence. In fact, in this method oocytes are typically retrieved from cycles which are nearly similar to natural cycles, then matured in vitro and fertilized by intracytoplasmic sperm injection. But reported implantation and pregnancy rates of this method are much lower than that achieved by IVF after controlled ovarian hyper stimulation (COH). The benefit of this method is that low hormonal stimulation is required, and there are fewer physical risks involved. Because IVM works best in patients with many antral follicles in the minimum stimulated ovaries, the procedure might have an even greater benefit to women with PCOS .Polycystic ovary syndrome patients, who have also the risk of recruitment of poor-quality embryos after COH/ICSI might benefit of IVM in another way. So, immature oocyte retrieval followed by in vitro maturation (IVM) opens a new horizon for modern assisted reproductive technologies (ART) and recent studies in IVM make it a feasible alternative to in vitro fertilization.

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K24 (Key Lecture 24): Reproductive tourism

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Reproductive tourism, where childless couples travel abroad to seek fertility treatment, is an increasing phenomenon, which serves couples in many ways. The reasons for seeking treatment in other countries are varied. Many countries simply do not have advanced IVF programs in place or do not allow it legally. Other reasons include lower costs of treatment and a desire to find sperm and egg donors of similar ethnic make-up as the infertile couple. Foreign couples also appreciate not having to wait long for an appointment, even if it means traveling to India. We, at our center, decided to retrospectively analyze the trend in the influx of patients coming from abroad, over a period of three years, from January 2004 to December 2006. The study included all overseas patients who had visited our center for treatments like IUI, IVF, ICSI, Donor Egg IVF and Gestational Surrogacy. Those availing of Surrogacy included patients with MRKH Syndrome, Asherman's Syndrome, those with history of Recurrent Pregnancy Loss, single parents, as well as lesbian and gay couples from abroad. In the year 2004, out of a total of 120 cycles, 9 cycles were done for patients coming from abroad (7.5%). Out of these 9 cycles, 3 (33.3%) cycles were of regular IVF, 1 of ICSI (11.1%) and 5 (55.6%) of Donor Egg IVF. In 2005, the percentage of cycles for patients from abroad increased to 10.6% (17/160). Of these, 5 (29.4%) were regular IVF cycles, 2 (11.8%) were ICSI, 7(41.2%) were Donor Egg IVF, 2(11.8%) were for Surrogacy and 1(5.8%) for IUI. In 2006, out of a total of 200 cycles, 30 (15.0%) were done for overseas patients. Of these, 7 (23.3%) were IVF cycles, 3(10.0%) were ICSI cycles, 11(36.7%) were Donor Egg IVF cycles, 7 (23.3%) were for Surrogacy and 2 (6.7%) were for IUI.Cheaper prices, high-quality health care and the availability of donor eggs and surrogates are drawing an increasing number of couples to Thailand, Eastern Europe, Russia, China and India. In the English-speaking world, India has a big advantage because of the availability of English-speaking doctors. The real benefit for many couples is relaxed laws. Whereas countries such as UK allow only two embryos to be transferred, in India this number is almost three times as much, increasing the chances of success in the first attempt itself. One of the biggest attractions offered by Indian ART clinics is maternal surrogacy. The Indian Council for Medical Research permits surrogates to claim monetary compensation in addition to routine expenses and antenatal care, a facility not provided for by many countries. This makes it easier for couples to find willing surrogate mothers in India. The number of surrogate births here has more than doubled in the past two years. Indian ART centers are also willing to treat women who have been deemed too old or overweight by the British National Heath Service (NHS) for IVF treatment. Consequently Indian clinics are performing a growing number of IVF treatments for foreigners frustrated with disappointing results and soaring costs at home.

K25 (Key Lecture 25): Uterus transplantation: A possible treatment for uterine infertility

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Transplantation of uterus is currently under investigation as a potential treatment for patients with uterine factor infertility. This type of organ transplantation has been investigated in mouse, rat, rabbit, dog, pig, and rhesus monkey. Also, a case of a human uterus transplant was reported in 2002. This was, however, terminated after 3 months, despite initial immunosuppression. Therefore, extensive experimental studies have been attempted to assess the mechanisms of uterine rejection, effective immunosuppressant, and the tolerability of the transplanted uterus to pregnancy. The method of transplantation involves a very complicated microsurgery, but has a high success rate in laboratory animal models. Following embryo/blastocyst transfer, successful implantation and delivery of healthy offspring has been reported in mice in 2007. The mechanism of rejection of the grafted uterus involves endometrium with an early invasion of leucocytes as illustrated by an increase in T lymphocytes. In general, the myometrium has the greatest resistance to rejection and retains its tissue structure for longer period of time. Collectively, the findings on the mechanism of uterine rejection patterns as well as the new development in immunosuppressant therapy along with the recent success in transplantation of the uterus in mice can serve as a model system towards development of uterine transplantation in human.

K26 (Key Lecture 26): New avenue in treatment of male fertility

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Remarkable advances have been made regarding the curative cancer treatments in pediatric and reproductive-aged male populations. Quality of life is increasingly important to long-term survivors of cancers. A major quality-of-life issue is the ability to produce and raise normal children. Surgery, radiation therapy, and chemotherapy may achieve relatively high remission and long-term survival rates, yet are often detrimental to male fertility. Radiation and chemotherapy are effective in cancer treatment by interfering with the more rapidly dividing cancer cells. However, the germ cells of spermatogenesis are similarly rapidly dividing and are thus, a target for these therapies. Cytotoxic therapy, received by men during childhood or adulthood, can lead to prolonged azoospermia or even sterility. Animal studies showed that spermatogonial stem cells can survive the challenge of cytotoxic therapy.Cryopreservation of sperm or testicular tissue is an effective method to preserve fertility prior to cancer treatment and in some cases after cancer treatments. For pre-pubertal boys whom fertility preservation through cryopreservation of semen is not possible, testicular germ cell harvesting provides a technique by which germ cells can be removed and store during cytotoxic therapy. At a later date, following recovery, the store tissue could be auto transplanted or these store cells could be mature in vitro until they reach a stage sufficiently mature to procure fertilization with assisted reproduction. In conclusion, autologous germ cell transplantation might become a clinically important technique to protect male germ cells during anti-cancer treatment to allow these patients to

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conserve their germ cell line *in vitro*. However, to develop a clinically applicable autologous germ cell transplantation program, techniques must be developed by which human spermatogonial stem cells can be isolated, stored and re-infused into the testis

K27 (Key Lecture 27): Towards economically feasible cell-based non-invasive prenatal diagnosis

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Fetal cells are present in the circulation of pregnant women, but they are extremely rare. As a consequence, fetal cells need to be enriched, their fetal character needs to be unequivocally confirmed and methods need to be developed for molecular genetic analysis based on single cells or very small cell pools. Techniques for cell enrichment using density centrifugation and/or cell sorting based on magnetic beads still result in a large number of cells which need to be screened for presence of markers characteristic for fetal cells. In order to reduce the cost of labor and to provide a perspective for cost-effective screening of pregnancies for fetal genetic health, we developed a method for automatic screening of cytospins of enriched fetal cells. On a model of anti-Hbε-FITC stained fetal erythroblasts (NRBC) from first trimester placental villi we established a classifier for semi-automated cell detection using the Metafer P module RCDetect (Metasystems). Automatic detection of NRBC was of the same quality as visual detection. Positive candidate cells were relocated and microdissected using the PALM Laser Pressure Catapulting (LPC) device. Currently DNA fingerprinting of isolated single cells is established, this will allow the confirmation of the fetal character of fetal candidate cells. Further plans include whole genome amplification and comparative genome hybridisation (CGH) on chromosomes.

K28 (Key Lecture 28): Stem cells from germ line linage

Aflatoonian, B[1]; Soleimani, M[2]; Aflatoonian, R[1]; Aflatoonian, A[1,2].

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Putative human embryonic germ (hEG) cells and mouse germline stem cells (mGSCs) are two sources of pluripotent cells derived respectively from primordial germ cells (PGCs) of the genital ridge of the human foetal in the first trimester gestation (5-9 weeks) and neonatal and adult mouse testis. These putative hEG cells and mGSCs are pluripotent which have potential to differentiate to other cell types of the body similar to embryonic stem cells, while they have the capacity of self-renewal themselves, suggesting that the germline lineage may maintain the ability to generate pluripotent stem cells.

It has reported that the expression of c-Kit mRNA and protein is germ cell specific in human foetal gonads. Also, c-Kit/ Kit Ligand (stem cell factor; SCF) signalling system has an important role in germ cell proliferation and survival in the developing human foetal gonad. Pluripotent stem cells from the germline lineage express molecular markers for pluripotency such as Oct4, Nanog, SOX2 and surface markers which are expressed in human embryonic stem (ES) cells such as

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TRA-2-54 and TRA-2-49 (Liver alkaline phosphatase), TRA-1-60, TRA-1-81, SSEA3, SSEA4, and SSEA1 (SSEA1 is not expressed in human ES cells).

Recently, in Yazd Clinical and Research Centre for Infertility, we have derived a cell line from human foetal gonocytes which has been characterised with c-Kit (a marker for gonocytes) and TRA-1-60 and TRA-2-49 (markers for hES and hEG cells) which we termed these cells, "embryonic gonocyte stem (EGS) cells". These putative EGS cells have been cultured in two different conditions (DMEM+ 10% FCS and standard HES medium; which is supplemented with bFGF and K/O Serum Replacement).

Mouse EG cells and mGSCs have the potential for entering meiotic. If further studies reveal that putative hEG cells and hEGS cells would have the same potential it might be applicable in the future to use these cells for treatment of infertility under the safe condition.

Another counterpart of pluripotent ES cells are embryonic carcinoma (EC) cells which are malignant stem cells of a germ cell tumour which reveals the serous consideration for the safety of the application of stem cells in cell therapy and clinics. Conversely, Germline stem cells have the potential for research on biology of cancers and ultimately cancer therapy.

During last 4 years, several reports have revealed that mouse and human ES cells and adult stem cells (bone marrow stem cells) have the potential to form gametes and in mouse these gametes from ES cells can make new offspring. These data from different reports has suggested that germ cells might be the mother of all stem cells which means possibly stem cells are originated from germline lineage cells.

A2a (Second award winner-shared): Expression of toll-like receptors in endometrium during the menstrual cycle

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Rapid innate immune defences against infection usually involve the recognition of invading pathogens by specific pattern recognition receptors recently attributed to the family of Toll-like receptors (TLR). Reports from our laboratory and others have demonstrated the existence of TLR1-6 in the female reproductive tract. However, little has been done to identify TLR7-10 in the female reproductive tract, particularly the uterus. In addition little information exists regarding variation in TLRs in the female reproductive tract during the menstrual cycle. In the current investigation, distribution of TLR7-10 protein was detected by immunostaining in timed endometrial biopsies from normal women. In addition total RNA was extracted after homogenization of tissue in Tri-reagent. Total RNA was treated with DNase I. cDNA was synthesized using Oligo dT primers and the Superscript II reverse transcriptase. RT-PCR was used to show the existence of TLR1-10 genes in endometrial tissue and real time PCR analysis to investigate relative expression of these genes during the menstrual cycle in normal human endometrium.The results demonstrated the existence of TLR 7, 8, 9 and 10 protein in endometrial tissue in human and showed that the mean relative expression of TLR genes 2, 3, 4, 5, 6, 9 and 10 was significantly higher during the secretory phase compared to all

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other phases of the cycle using Quantitative PCR. There was at least a two fold difference in the relative expression of these genes between the secretory and proliferative phases of the cycle. The relative expression of TLR 1, 7 and 8 genes did not significantly change during the menstrual cycle in endometrial biopsies. Our future studies are directed towards understanding the mechanism of cycle dependent expression of TLR genes and their role in regulation of innate immunity in the female reproductive tract.

A2b (Second award winner-shared): Developmental and ultra structural studies of zygotes derived from reconstructed oocytes of old mice using nuclear transfer and activated sperm

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The aim of this research was to study the Developmental and ultra structures of zygotes derived reconstructed oocytes of old mice using nuclear transfer and activated sperm and then compare the ultra structure with old and young zygotes. MII oocytes were retrieved from old NMRI mice (7-9 months old) and young NMRI mice (10-12 weeks old) after ovarian stimulation. Spermatozoa were collected from the cauda epididymidis NMRI male mice (10-12 weeks old). Sperms were added to KSOM medium containing MII young and old oocytes and incubated at 37ċ under 5% co2 in air. For formation of zygotes derived reconstructed oocytes , the MII oocytes meitotic apparatus (karyoblast) from old mice with activated sperm head (with ca^{+2}) was transferred into an enucleated metaphase II oocyte from young mice using piezo-actuated injection. Survival and fertilization rate were evaluated after 4-5 hours incubation. The micro manipulation medium for old oocytes was KSOM containing 3% (w/v) sucrose, 20% FCS and 7.5 µg/ml Cytochalasin B while the medium for determination of MII chromosomes of young oocytes was contained 5µg/ml hoechest 33342 (sigma) instead of sucrose. 2PN and 2cell embryos (5 zygotes in each group) were fixed and processed for TEM analysis. Old karyoblasts were extracted and injected with head of activated sperm to 525 young enucleated oocytes (YEO) and with head of normal sperms to 215 YEO. There was no significant difference between two groups (activated and normal sperm) in fertilization rate. The results are summarized in table I:

No. of MII old group	No. of MII young group	No. of YEO	No. of survived young Oocytes 1h after enucleation	No. of YEO used for old oocyte nuclear transfer	No. of survived reconstructed Fertilized oocyte	No. of reconstructed Embryos develop until 2pn
1280	3710	3200	1728 (54%)	525 (Ca-I)	124 (24%)	51 (41%)
				215 (normal)	50 (23%)	20 (40%)

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Embryos driven from young, old, reconstructed oocytes were surrounded by zona Pellocida. Subzona space was seen in three groups. Polar body and cellular fragmentation were seen in subzona space. Perivitellin space in old and reconstructed zygotes was expanded and microvillies were arranged regularly. Nuclei of three groups were euchromatin. In old and reconstructed embryos, intranuclear anulatelle lamellae were seen. Mitochondria and intermediate filament were seen in three groups in cytoplasm but their arrangement and distribution were different. In old embryos, most of mitochondria were central and around the nucleus, while in young and reconstructed embryos were distributed in cytoplasm. SER collection was higher in young, reconstructed embryos while residual bodies and lipid were more frequent in old embryos. In reconstructed embryo, relation was seen

between the cytoplasm and transfer karyoblast. One of the reasons of low fertilization and implantation rate in old infertile couples can be several ultra structural changes in embryos driven from old oocytes in comparison to young embryos. However fertilization rate of reconstructed embryos was low, improved nuclear transfer technique can be a method for helping old infertile couples.

A3a (Third winner, shared):
Localization and variable expression of Gai2 in human endometrium and Fallopian tubes

Mönkkönen, K; Aflatoonian, R; Lee K.F; Yeung, W.S.B; Tsao, S.W; Laitinen, J.T; Tuckerman, E.M; Li, T.C; Fazeli, A

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Heterotrimeric G proteins take part in membrane mediated cell signaling and have a role in e.g. hormonal regulation. This study clarifies the expression and localization of the G protein subunit $G\alpha_{i2}$ in the human endometrium and fallopian tube and changes in $G\alpha_{i2}$ expression in human endometrium during the menstrual cycle. The expression of $G\alpha_{i2}$ was identified by PCR, and localization confirmed by immunostaining. Cyclic changes in $G\alpha_{i2}$ expression during the menstrual cycle were evaluated by quantitative real time PCR.. We found $G\alpha_{i2}$ to be expressed in human endometrium, fallopian tube tissue and fallopian tube primary epithelial cells. Our studies revealed enriched localization of $G\alpha_{i2}$ in human fallopian tube cilia and in endometrial glands. We showed that $G\alpha_{i2}$ expression in human endometrium changes significantly during the menstrual cycle. $G\alpha_{i2}$ is specifically localized in human fallopian tube cilia and is likely to have a ciliaspecific role in reproduction. Significantly variable expression of $G\alpha_{i2}$ during the menstrual cycle suggests it might be under hormonal regulation in the female reproductive tract *in vivo*.

A3b (Third winner, shared):
The expression of prostaglandin endoperoxide synthase 2 messenger RNA and the proportion of smooth muscle and collagen in the sheep cervix during the estrous cycle

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The use of transcervical artificial insemination in sheep is limited because of the anatomy of the cervix, which restricts the passage of an inseminating pipette into the uterine lumen. There is a degree of natural cervical relaxation at estrus that enables greater penetration with an inseminating pipette. We hypothesize that this relaxation may be regulated by cervical prostaglandin synthesis and remodeling of the cervical extracellular matrix. The present study investigated the changes in prostaglandin endoperoxide synthase 2 (PTGS2) mRNA expression and the proportion of smooth muscle and collagen in the sheep cervix during the estrous cycle. Sheep cervices were collected at four stages of the estrous cycle: prior to the LH surge, during the LH surge, after the LH surge, and during the luteal phase. The expression of cervical PTGS2 mRNA was determined by in situ hybridization, and the proportion of smooth muscle and collagen in the cervix was investigated by Masson trichrome staining. The expression of PTGS2 mRNA in the sheep cervix was greatest prior to the LH surge, when estradiol concentrations were also greatest. The increase in PTGS2 mRNA expression was associated with an increase in the proportion of collagen in the sheep cervix. We propose that prior to the LH surge, estradiol may stimulate PTGS2 mRNA expression and hence prostaglandin E2 synthesis in the sheep cervix to regulate cervical relaxation, most likely through the rearrangement of collagen bundles within the cervical extracellular matrix.

A4a (Forth winner, shared):
In-vitro maturation for infertile women with PCOS

Eftekhar, M; Karimzadeh, M.A ;Tayebi, N

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Objective: In vitro oocyte maturation (IVM) allows the use of immature oocytes in ART. In this procedure, immature oocytes are retrieved from unstimulated or minimal stimulated and therefore can be offered to patients at risk of ovarian hyperstimulation syndrome. IVM can be offered as an alternative to conventional IVF in women with PCOS (polycystic ovarian syndrome).Patients: In this study, we will carry out 16 cycles of IVM in 16 women (18-40 years) with polycystic ovarian syndrome (PCOS). Methods: Patients received minimal ovarian stimulation by FSH 75Iu/day (Cycle day 3-8) and then received HCG 10000 IU on day 8 of cycle. oocyte retrieval was performed on day 10 of cycle. Immature oocytes transferred to IVM culture media. Oocytes were checked since 24h after oocte retreived for maturation and mature oocytes were fertilized by ICSI.Main Outcome Measure(S): Immature oocytes collected, metaphase II oocytes, and embryos produced. Implantation and pregnancy rates. Results: In this study, the mean number of immature oocytes retrieved was 4.31 per cycle . Maturation and fertilization and chemical pregnancy were 91%,76% and 12.5% respectively.

A4b (Forth winner, shared):
Comparison of coasting with cabergoline administration for prevention of severe OHSS in ART cycles.

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One of the major and life-threatening side effects of Assisted Reproduction Technique (ART) is Ovarian Hyperstimulation Syndrome (OHSS). To avoid Cycle cancellation, while maintaining excellent PR and diminishing the risk of severe OHSS, Coasting has been advocated. Alvarez study showed that the administration of Cabergoline reduces the severity of OHSS. In this study, we compared coasting and administration of Cabergoline (anti VEGF) in prevention of severe OHSS. 44 women underwent ART treatment cycles in 2006-2007 who were at high risk for developing OHSS (≥ 20 follicles in both ovaries and most of the follicles had a diameter > 15mm and serum estradiol $E_2 \geq 2500$ pg/ml) were randomly divided in two groups. All patients underwent induction of ovulation with long protocol of gonadotropin stimulation beginning by pituitary desensitization with subcutaneous buserelin and followed by hMG 2-3 Amp from day 2 cycles. Follicular monitoring was performed on day 7 .Group I (cabergolin group) consisted 23 patients of IVF or ICSI cycles at risk of developing OHSS . These patients received 0.5 mg Cabergoline on the day of HCG administration for eight days. In group II (Coasting) 21 patients with the same criteria, Gonadotropins were withheld until the serum E2 levels started to decline. Embryo transfer procedures in both groups were carried out in a similar fashion. These results of 44 patients have listed .The mean number of oocytes retrieved; Metaphase 2 oocytes and good quality oocytes were higher in group 1. The mean number of embryos available was higher in group 1 than in group 2. The quality of embryos was similar in both groups. The incidence of severe OHSS was similar in both groups. CPR was higher in group 1 than group 2 but not significantly.

A5a (Fifth winner, shared):

The effect of pregnant mouse serum on IDO induction in dendritic cells

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Pregnancy as a strange and complicated phenomenon has stimulated many investigations on maternal tolerance to semi-allogeneic fetus. Many factors seem to be involved in this tolerance. IDO enzyme expression at the feto-maternal interface is one of these factors which have recently attracted too much attention, so that, the inoculation of enzyme inhibitor caused abortion in allogenic pregnancies. Since DCs are one of the most important producers of IDO enzyme, this study was done to assess the pregnant mouse serum effect on IDO enzyme induction in DCs. Allogenic pregnant mice sera (Balb/c × C57BL/6) were collected in mid-pregnancy (day 9-11). DCs were isolated from Balb/c mouse spleen through a three step method including: collagenase digestion of spleen tissue, low density cells separation via the Nycodenz density gradient centrifugation and plastic adherence. The purity of DCs was determined through flow cytometric method, using anti-mouse CD11c Ab. The T cells were isolated from C57BL/6 mouse lymph node through Nylon wool method and their purity was determined using anti-mouse CD3 antibody. As stimulator cells, pregnant and non-pregnant mouse serum treated DCs were irradiated and co-cultured with purified T cells (allogenic MLR). 1-methyl- tryptophan (1-MT) as the

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specific inhibitor of IDO, was added to some wells of MLR assay in different concentrations and the T cells proliferation response was measured by ^{3}H-thymidine incorporation. The MLR supernatant was also analyzed by HPLC for its tryptophan and kynurenin (Trp metabolite) content. Our results showed the ability of pregnant mouse serum to reduce the dendritic cells ability in T cell proliferation induction when compared to non-pregnant mouse serum, but addition of 1-MT did not show any significant effect on this inhibition. Additionally, IDO metabolite's concentration assessment in presence and absence of 1-MT, through HPLC method, did not show any significant differences. There are many factors in pregnant mouse serum such as IL-10, HLA-G, progesterone, etc which might have caused inhibition of T lymphocyte proliferation response in allogenic MLR through, affecting DCs efficiency. IDO specific inhibition did not induce any significant increase in T cell proliferation response and we did not see any significant difference between supernatant of pregnant serum treated and non-pregnant serum treated groups regarding their Trp metabolite (Kynurenin) concentration. So it seems that IDO expression by DCs is not responsible for decrease in T cell proliferation after treatment of DCs with pregnant serum and some other mechanisms are responsible for this phenomenon which their identification needs more investigations.

A5b (Fifth winner, shared): Biphasic effects of Atorvastatin on cultured human endometrium in three-dimensional fibrin matrix

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Angiogenesis has been proposed as an important mechanism in the pathogenesis of endometriosis and HMG-CoA reductase inhibitors (statins) have been shown to have anti-inflammatory and anti-angiogenic activity. The aim of this study was to investigate the effect of atorvastatin on cultured human endometrium in three-dimensional fibrin matrix. Endometrial samples obtained from 8 normal ovulating women undergoing surgery for the various benign gynecological indications, were cultured in fibrin matrix for 21 days. Degree of cell proliferation and invasion of the fibrin matrix and the presence or absence of gland and capillary-like sprouting were detected. The results demonstrate that during the 1st week of culture, invasion of stromal cells and cell outgrowth arising from the main endometrial fragments into fibrin matrix were observed in some control wells. After 2 weeks, glandular-like structures were observed in some control wells and after 3 weeks, cell outgrowths and capillary-like structures consistent with angiogenesis were detectable in 82.5% of control wells. A dose-dependent effect of atorvastatin was seen on cell growth and angiogenesis in the experimental groups. In the presence of 1μM and 10μM atorvastatin, cell proliferation and angiogenesis was reduced significantly ($p<0.001$) compared with the control wells. In the presence of 0.1 μM atorvastatin, angiogenesis was increased significantly ($p<0.001$) compared with the control wells. This study reports that HMG-CoA reducatse inhibitor and atorvastatin, has a biphasic dose dependent effect on angiogenesis and cell proliferation in an experimental model for the development of endometriosis-like tissue.

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O1 (oral presentation 1): Cytotrophoblast stem cell lines derived from human embryonic stem cells and their capacity to mimic invasive implantation events

Harun, R; Ruban, L;Matin, M; Draper, J;Jenkins, N.M; Liew, G.C; Andrews, P.W; Li, T.C;Laird, S.M and Moore H.D.M.

Centre for Stem Cell Biology, University of Sheffield. National Population and Family Development Board, Ministry of Women, Family and Community Development, Bangunan LPPKN, 12B Jalan Raja Laut, Peti Surat 10416, 50712 Kuala Lumpur, Malaysia

An effective embryonic–maternal interaction is crucial for successful human pregnancy. Failure of this process is a major cause of infertility and can lead to placental dysfunction resulting in recurrent miscarriage, fetal retardation and pre-eclampsia. Research is severely constrained by ethical and practical considerations; therefore, we aimed to generate cytotrophoblast stem (CTBS) cell lines from human embryonic stem cells (HESCs). b-HCG was used as a marker of viable trophoblast cells. In defined culture, embryoid bodies were generated from HESCs and selected for trophoblast enrichment by rounds of cellular aggregation and disaggregation. Distinct CTBS cell lines were isolated and characterized. Spheroid cytotrophoblast bodies were generated and their interaction with lutealphase endometrial stroma was analyzed by real-time image analysis. Three CTBS cell lines were derived, which were maintained in the absence of residual HESCs, fibroblast feeder cells or extracellular matrix. CTBS cells displayed typical cytotrophoblast and syncytiotrophoblast characteristics and exhibited further differentiation to invasive endovascular cell phenotype. One cell line was generated with constitutive expression of enhanced green fluorescent protein (eGFP). Spheroid trophoblast bodies mimicked closely the early invasive stages of implantation when incubated with human endometrial stromal preparations *in vitro*.These human CTBS cell lines are a significant new model for investigating human placentation and may have considerable potential in cell therapy applications.

O2 (oral presentation 2): The effect of co-culture system and erythropoietin on differentiation of embryonic stem cells into erythroid colonies and the ability of the differentiated cells on colony formation in-vivo.

Beigi Boroujeni, M;Salehnia, M; Rezazadeh Valojerdi, M; Mowla ,S.J; Forouzandeh, M.

Tarbiat Modarres University, Tehran, Iran.

The feeder layer and some growth factors could have some inductive effect on differentiation of embryonic stem cells (ESCs). The differentiation capacities of embryonic stem cells (ESCs) make them a promising source of stem cells for transplant therapies. Erythropoietin (EPO) is one of the factors that have a role in differentiation of ESCs into erythropoietic cells. The aim of this study was to evaluate the effect of erythropoietin in simple and co-culture systems on differentiation of ES cells into erythroid colonies in-vitro. This study also focused on homing of EPO-differentiated embryonic stem cells in hematopoietic organs after transplantation. Embryoid bodies were dissociated and replated in semisolid medium containing different doses of EPO (0, 10, 20, 40, 80, 160 ng/ml) in simple culture system. The best dose of EPO was selected among different experimental doses following colony assay. In simple culture and co-culture

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systems (with bone marrow stromal cells) in the presence or absence of EPO colony assay was carried out up to10 days. The benzidine staining and giemsa staining were done for confirmation of erythroid colonies. The expression of ε, βh1, AML1, β Major and EPOR genes were evaluated using semi quantitative RT-PCR. For in-vivo study the cells of four days old embryoid bodies were dissociated and replated in Iscove's modified Dulbecco's medium supplemented with FBS (15%), monothioglycerol (4.5×10^{4} mol/L), ascorbic acid (12.5 ng/ml), L-glutamine (2 mmol/L) and 20 ng/ml of EPO for three days. The experiment without EPO consider as control. Cells were labeled with 10 µg/ml 5-bromo-2 deoxyuridine (Brdu) before transplantation and injected into (2×10^{6}) of sublethally irradiate mice via tail vein. The animals were killed 10 days after injection and spleen colony assay were done. Paraffin sections of spleen and liver were prepared and immuno-histochemistray procedure was carried out for Brdu. Comparing with the corresponding controls showed that the size of colonies were increased in both systems ($P \leq 0.05$) and number of benzidine positive colonies in the co-culture system with EPO (86.6 ± 17.89) had significant difference compared to simple culture system (45.6 ± 4.77; $P \leq 0.05$). Analysis of gene expression showed that all genes except βMajor were expressed in simple culture system. However in case of co-culture system all genes were expressed. Also in-vivo results showed that the number of colonies formed in spleen of experimental group (17.33 ± 4.726) had significant difference with control group (6 ± 1; $P \leq 0.05$). No colonies were observed in spleen of sham group. Also Brdu positive cells were observed in sections of spleen and central vein and disse space of liver. Our results confirmed that the presence of EPO in co-culture system of BMSCs with ES cells gave better results than the simple culture system for improving the differentiation of ES cells to erythroid colonies. It seems that both primitive and definitive erythropoiesis had occurred in this co-culture system and ESCs were treated with EPO have ability to homing and forming colony in spleen after transplantation and it will be an alternative for cell therapy in future.

O3 (oral presentation 3):
Proteomic Analysis of pig oviductal epithelial cells at different stages of the reproductive Cycle

Seytanoglu, A

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The mammalian oviduct is the site of crucial events leading to the establishment of the pregnancy. The oviductal epithelial cells are the main sites of contact with both gametes during these events. We isolated oviductal epithelial cells from pig oviducts in follicular and luteal stages. We used a combination of two-dimensional gel electrophoresis and liquid chromatography-tandem mass spectrometry to identify differences in the cell proteome during the different stages of the reproductive cycle. We found 51 upregulated and 27 downregulated proteins during the follicular relative to luteal phase. We identified nine of the upregulated and five of the down regulated proteins. The findings show that the oviductal cell proteome is altered during the reproductive cycle. Attempts were made to quantitatively identify changes to the proteome occurring specifically at the cell surface of the oviductal epithelial cells using isobaric tagging.

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Oviductal cell-surface proteins from follicular and luteal cells were successfully purified. Work is ongoing to characterize changes occurring at the oviductal cell-surface during the reproductive cycle.

O4 (oral presentation 4):
Islamic perspectives and sectarian implications in the practice of assisted reproductive technology (ART)

Fraouk, M.

Infertility is a common affliction and has been the seed of discord in marriage, family and society. Recent advances in molecular biology, genetics and reproductive medicine have opened up exciting and promising technologies in the field of ART; the scope for the barren has been extended to even those without sperm, egg or uterus! The above benefits notwithstanding, ART has given rise to ethical, theological and legal concerns with Muslims and their Semitic kinsmen being denied some of the potential benefits of modern technology in reproductive medicine. Closer scrutiny of the Shari'a (Islamic law) may permit the use of some ARTs, which has hitherto been proscribed. There is confusion and uncertainty among patients, ART specialists and Islamic scholars regarding the Shari'ah status of some ART modalities. This and divergences in Shari'a interpretation among the Sunnis and the Shi'ah needs more enquiry and research. To review current developments and Islamic arguments for and against controversial ARTs and clarify the ambiguity and uncertainty prevailing among professionals and the Islamic community. The research methodology consists of a literature review and a survey component consisting of a cross-sectional, mainly qualitative, semi-structured research supported by secondary analysis of key surveys in the field. A survey of opinions and perceptions of those intimately involved in ART, the patients, physicians and Islamic scholars has been conducted. The patients and Muslim ART specialists were enthusiastic; while the Sunni scholars were more conservative, their Shi'a counterparts were more pro-active. While some ARTs pose no problems for Islam and others whose proscription has been solved by the newer methodologies, there are still several which defy Shari'a compatibility. Recent Shi'a ijtihadi approaches have provided an avenue which may help solve this problem.

O5 (oral presentation 5):
Effects selenium on sperm parameters and CatSper expression in aging mouse testis

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CatSper1 through CatSper4 are a family of specific-sperm calcium channels that expressed exclusively in the testis and related to sperm motility and male fertility. The literature implicates Selenium (Se) is essential element for sperm motility and it affect gene expression in mammalian cells. Also most studies show a decrease in sperm motility with increasing age. So in this research we investigated the effects of Selenium on expression of CatSper genes and sperm parameters in aging male mice. Forty aging male mice 10-12 months and forty adult male mice 2-3 months were used in this study (exp.1: aging male mice treated with Selenium, exp.2: adult male mice treated with Selenium). Experimental groups of male mice were injected

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intraperitoneally Se (Na_2Seo_3, 0.2 mg/kg daily). Se treatment was followed daily for 5 weeks. Animal sacrified was carried out by cervical dislocation at days 21, 28, 35 and 42 after treatment. Testes were collected from each group. One of the testes was snap frozen for extraction of total RNA. RT-PCR reaction was performed for both CatSper and β2m genes. After gel electrophoresis, intensity of each band was quantified. Another test was fixed in Bouin's for histological examination. The testicular tissues were paraffin-embedded, cut (4µm thick) and stained with hematoxylin and eosin. For sperm parameters (count, motility, morphology and viability rates of sperm) studies, a sperm aliqute was prepared from epididymis in Phosphate Buffer Saline (PBS).We used immunohistochemistry to determine the location of the protein CatSper in sperm. Also, Total Antioxidant Capacity measured in sperm using FRAP (Ferric reducing ability of plasma) method. The data were analyzed by ANOVA test and was considered significant when p values were < 0.05. The expression of CatSper genes was evaluated by semi-quantitative RT-PCR. Results revealed that there are significant up-regulation for experimental groups comparing control ones. Selenium treatment in aging male mice caused more CatSper expression comparing adult ones. Sperm parameters analyzed according to WHO guidelines for the examination of human sperm. The results of sperm analysis showed that sperm parameters improve in aging and adult male mice following of Se treatment comparing of control group. Results of immunohistochemistry staining showed that CatSper protein was localized in principal piece of sperm tail. Conclusion: In conclusion, Se treatment in aging subjects can up regulate CatSper gene expression which is one of the responsible genes of producing sperm motility. Beside, Se treatment improve sperm parameters especially morphology rate.

O6 (oral presentation 6):
Autograft of mature mouse spermatogonial stem cells, after *in vitro* treatment with SCF, GM-CSF, GDNF, into azoospermia mouse model generated by gamma irradiation

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Spermatogenesis is a complex process involving formation of a huge number of cells that eventually differentiate into sperm. The basis of this process is the spermatogonial stem cells (SSCs). Despite their importance in infertility treatments and others studies, SSCs are infrequent in adult mouse testis and their proliferation is low. In other hand, culture techniques, cryopreservation, and transplantation are useful tools for the better understanding male germ cell biology and regulatory factors of male fertility. In the present study, we examined the autograft of mature mouse spermatogonial stem cells, after in vitro treatment with (Stem Cell Factor) SCF, (Granulocyte Macrophage-Colony Stimulating Factor) GM-CSF, (Glial Cell line-derived Neurotrophic Factor) GDNF, into azoospermia mouse model generated by gamma irradiation. After preparation of azoospermic model testis with ^{60}Co γ-ray from cobalt therapy machine (Shohada-E-Tajrish Hospital) and improvement culture condition with co-culture system, GDNF, SCF and GM-CSF, right testis from adult 6-8 weeks old NMRI mice were collected for cell suspension. Sertoli and

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spermatogonial cells were isolated from adult mice testes using two step enzymatic digestion and lectin immobilization. After identity confirmation of isolated cells, spermatogonial cells of resulted colonies from right testis were transplanted into the seminiferous tubules of another testis of the same mouse which were irradiated with 14Gy at 10 weeks of age, via rete testis. They were transplanted into 4 groups: fresh cells (control 1), fresh cells co cultured with sertoli cell (control 2), frozen-thawed SSCs (experimental 1) and frozen-thawed SSCs co-cultured with sertoli cells (experimental 2).The statistical significance between mean values was determined using repeated measure ANOVA test; $P<0.05$ was considered as significant level. According to results of this study: 1) ^{60}Co γ-ray with dose of 14Gy caused temporary azoospermia in mouse. 2) Co-culture system with sertoli cells showed a significant increase in number and diameter of colonies compared with growth factors treated and control groups. 3) Co-culture system with Sertoli cells could increase *in vitro* colony formation of adult fresh and frozen-thawed spermatogonial cells. 4) Transplanted spermatogonial cells 8 weeks after autologous transplantation resulted proliferation and sperm reproduction in the recipient testes. There was a significant increase in epididimis sperms counts, following transplantation. In this study; we demonstrated that co-culture system with Sertoli cells can increase *in vitro* colony formation of adult fresh and frozen-thawed spermatogonial cells. Also, autograft of mature mouse spermatogonial stem cells can increase fertility rate in azoospermic mice.

O7 (oral presentation 7):
Mutations in Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPX) gene in Iranian infertile men

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Leukocytes and defective or/and dead spermatozoa in human semen are a source for production of reactive oxygen species (ROS) and subsequent injury to intact sperms. Enzymatic and nonenzymatic defensive mechanisms in semen detoxify these compounds. Glutathione peroxidase-4 (GPX-4 or PHGPX) is a major selenoprotein in sperm. Function of this protein is one of the enzymatic mechanisms that play multiple roles during spermatogenesis such as formation of the mitochondrial capsule, hydroperoxide detoxification and sperm chromatin condensation. Any decrease in the enzyme activity or content, may create disorders in spermatogenesis and sperm fertilizing ability. Considering Defect in expression of the enzyme gene or presence of mutations may cause decrease in PHGPX activity or content, this study was carried out to identify a number of important mutations in gpx-4 gene by PCR-RFLP method in Iranian infertile men. This study was performed on 128 Iranian male participants who had been referred to Avesina Infertility Clinic, including 74 infertile men with defective sperm parameters, 18 normozoospermic and 36 fertile subjects as controls. Mean ± SD for sperm parameters were determined using SPSS (version 13). Genomic DNA was extracted using salting out procedure from peripheral blood leukocytes. PCR-RFLP was done by two sets of Primers with 237 bp and 148 bp PCR products, that were designed for 1A and 4 exons of gpx-4

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gene covering nucleotides of +6 (C→T), +17 (G→A), +1725 (G→A).Digestion of a 237 bp intact PCR product with MWOI generates two pieces (151 bp and 86 bp). When a mutation occurs in the restriction site +6 (C→T), the enzyme will not recognize the sequence, therefore 237 bp segment remains undigested. Treatment of 237 bp segment with PshAI generates two fragments (161 bp and 76) in the intact gene but the same enzyme can not digest 237 bp segment when a mutation occurs in the restriction site +17 (G→A). Ultimately, Digestion of 148 bp intact segment with SatI generates two fragments (108 bp and 40) but when a mutation occurs in the restriction site 1725(G→A), the enzyme will not recognize the sequence; therefore 148 bp segment remains undigested. Evaluation of enzymatic digestion on 237 bp and 148 bp segments in all participants revealed that neither of the examined mutations existed in gpx-4 gene. According to the results of this study, it was determined that the prevalence of these mutations in Iranian infertile men is probably low and it may have no association with the etiology of the disorder in sperm parameters. Hence, a study with a larger number of patients is suggested to determine the exact prevalence of these and other mutations in this gene in Iranian infertile men.

O8 (oral presentation 8):
Immobilization effect of Ruta graveolens L. on human sperm: A new hope for male contraception

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Being both an ancient and a modern concept, male contraception is an interesting matter of investigation. Contraceptive plants which were introduced by folk in traditional remedies are investigated worldwide. In this study, the contraceptive effects of Ruta graveolens L., which has been mentioned for male contraceptive in Iranian traditional folk medicine, was experimented on human sperm Different doses of lyophilized aqueous extract of Ruta graveolens L. were added to an amount of fresh semen, containing 10^6 cells in a 1:1 volumic ratio. To unravel the mode of Ruta graveolens L. action, motility and viability of cells, DNA status, mitochondrial activity and sperm revival tests were carried out. The sperm immobilization effects of the extract appeared immediately in a does-dependent manner and 100% of the sperms became immotile at a concentration of 100 mg/ml but viability of cells, DNA status and mitochondrial function were intact. After washing the sperms twice with phosphate buffer saline solution, motility was observed in 30.8 ± 3.2% of the sperms, besides coiled tails in 38.6 ± 5.5% of the treated cells, in comparison to 12.5 ± 2.0 % of the control group ($p = 0.001$). The part of the extract, responsible for immobilization of the sperms was stable upon boiling. As the cells were alive and immotile, probably some ionic currents are blocked by a thermo stable component of the plant which can be promising as a new male channel blocker contraceptive.

O9 (oral presentation 9):
The Association of Seminal Inflammatory Markers with *C. trachomatis* Infection in Male Infertility

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Kokab A, Eley A.R, Akhondi M.M, Sadeghi M.R, Modarressi M.H, Aarabi M, Jennings R, Pacey A.A.

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Chlamydia trachomatis infection is the most common bacterial sexually transmitted infection throughout the world. In females infections may lead to tubal factor infertility, pelvic inflammatory disease, salpingits and ectopic pregnancy. In men, it is associated with a wide clinical spectrum which may lead to infertility. Both males and females can suffer asymptomatic infection. C. trachomatis infections can be transmitted from either symptomatic or asymptomatic males or females to the opposite sex. The way to diagnose these symptoms less infections is by laboratory testing. The aim of this study was to evaluate the relationship between the presence of Chlamydia trachomatis and inflammatory markers in semen from males of infertile couples. Concentrations of leukocytes, interleukin (IL)-8 and IL-6 were determined in seminal plasma (SP) from 255 male partners of asymptomatic infertile couples undergoing diagnostic semen analysis as part of ongoing infertility investigations. Semen analysis was performed according to WHO (1999) methods. In addition, strand displacement amplification (SDA) and polymer conjugate enhanced enzyme immunoassay (IDEIA PCE) were undertaken on semen and first-void urine samples to detect the presence of C. trachomatis-specific DNA and Chlamydia genus-specific antigen respectively. Nested plasmid PCR and Direct Immunofluorescence (DIF) were used to confirm positive SDA and EIA. Sperm motility and viability were assessed both initially and after three hours incubation at 37°C. A total of 14 men (5.5%) were found to meet our criteria of genital Chlamydial infection by having at least two positive samples (semen and urine) and/or two positive tests (SDA and IDEIA PCE) for Chlamydia. Men with Chlamydial infection had a significantly ($P<0.05$) higher level of IL-8 and higher mean concentration of leukocytes present in their SP, than those without infection. The group without infection also had a significantly ($P<0.05$) greater percentage of progressive motile sperm. The SP concentration of IL-6 was much lower than IL-8 levels and there was no significant correlation with Chlamydial infection. There was a degree of correlation between IL-6 and IL-8 in SP of studied males. These results suggest that raised seminal IL-8 might be useful as a marker for silent male genital Chlamydial infection with in turn is associated with decreased sperm motility.

O10 (Oral presentation 10): Evaluation of an existing nomogram for predicting the response to clomiphene citrate

Ghobadi, C; Nguyen, T.H; Lennard, M.S ;Amer, S; Rostami-Hodjegan, A and W.L Ledger,.Phil D.

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To evaluate the ability of an existing nomogram to predict response to clomiphene citrate (CC) in infertile couples with World Health Organization group II ovulatory

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disorders, using the free androgen index, body mass index, and menstrual-cycle history. Retrospective case-notes study. Reproductive medicine and fertility center at a university teaching hospital in the United Kingdom. One hundred and four anovulatory women. One hundred four anovulatory women who had been treated with CC were studied retrospectively. Age, body mass index, free androgen index, and cycle history were used to assign a likelihood of response for each patient on the basis of a published nomogram. Predicted and observed responses were compared. Ovulationrate. The diagnostic characteristics of the model on the basis of the optimal cutoff points were as follows: sensitivity, 96% (95% confidence interval [CI]: 90%–99%); specificity, 33% (95% CI: 18%–49%); positive predictive value, 73% (95% CI: 63%–82%); negative predictive value, 80% (95% CI: 60%–99%); likelihood ratio for ovulation, 1.34 (95% CI: 1.1–1.8); likelihood ratio for resistance, 0.13 (95% CI: 0.04–0.43); _, 0. 26 (95% CI: 0.09–0.44). The accurate prediction of response to CC would allow more rapid transfer of nonresponders to alternative treatments and may shorten the treatment to pregnancy interval. Although the current nomogram could identify 80% of nonresponders to CC, it was insufficiently accurate for use in the present clinical setting. Moreover, the nomogram could not identify the most appropriate dose to achieve ovulation. This nomogram should be tested on patients in other clinical settings, where it may perform better.

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P1 (Poster presentation 1):
Role of FSH receptor gene polymorphisms in ovarian response to stimulation in patients entering IVF/ICSI programs

Sheikhha, M.H; Eftekhar, M;Tayebi, N; Fazli, H; Kalantar, S.M.

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Objective: To examine the frequency distribution of the Ser680Asn polymorphism of the follicle-stimulating hormone receptor gene in infertile women" poor responder, good responder and OHSS. Methods: sixty infertile women participated in this study. The hormonal profiles and treatment of all patients were analyzed and FSHR polymorphism was examined by PCR. Women from all groups were classified as Asn/Asn(AA), Asn/Ser(AS), and Ser/Ser(SS) genotypes. Results: The frequency distribution of AA and AS was 21 (19.4%) and 87(80.6%) respectively. SS genotypes were not detected in this study. The mean age of women in AA and AS groups was 29.95±4.9 and 29.89±4.4 years respectively (P-value=0.7). The mean of total follicles were 13.38±9.4 and 11.92±8.9 in AA and AS genotypes respectively (P-value=0.52). The mean number of oocytes were 5±3.8 and 4.5±4.1 in AA and AS genotypes respectively (P-value=0.62). In the AA group, the mean basal level of serum FSH was 5.5 ±2.1 and in the AS group, it was 6.7±3.6 (P-value=0.053).Conclusion: These results suggest that FSH receptor polymorphisms have no effect on FSH receptor in ART cycles.

P2 (Poster presentation 2):
Age and basal follicle stimulating hormone as predictors of ART outcome

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With increasing age the probability of pregnancy established by the use of ART decreases. Also, many cycling women with elevated basal FSH level have been discouraged from undergoing ART treatment. This is because elevated basal FSH is associated with poorer ART outcome. The aim of this study is to assess the effect of parameters of ovarian functional reserve including female age and basal FSH level on the results of ART. The study was a prospective analytic investigation of two hundred women undergoing the first cycle of ART and they haven't had any pelvic surgery. In this study, basal (menstrual- day 3) FSH concentration will be measured and the woman's age calculated before she is undergoing pituitary desensitization followed by gonadotrophin ovarian stimulation and ART treatment. From 200 patients, 120 of them have been gathered until now. Increasing age was associated significantly with increased total gonadotrophin dose. Increasing basal FSH concentration was associated significantly with reduced number of oocytes and follicles and embryo collected. Both increasing FSH and age had significant association with cancellation rate.

P3 (Poster presentation 3):
The effect of progesterone and exogenous gonadotropin on preimplantation mouse embryo development and implantation

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The aim of this study was to evaluate the effects of progesterone and ovarian stimulation on the development and implantation rate of mouse embryos. Two cell embryos were collected from superovulated mice and cultured in the presence of different concentrations of progesterone (0, 5, 10 and 20ng/ml). Also other groups of mice were rendered pregnant in non-stimulated, non-stimulated progesterone treated, superovulated, superovulated-progesterone treated groups to collect the blastocysts. The number of blastocysts and implantation site were recorded on day fourth and 7th of pregnancy respectively. The diameter and cell number of blastocysts were analyzed in vitro and in vivo groups. The 60% of two-cell mouse embryos were reached to blastocyst stage in medium with 20ng/ml of progesterone after 72 h of culturing and it was higher than other groups ($P<0.001$). *In vivo* survival rate of blastocyst (97.68%) and implantation rate (92.06%) in non-stimulated and progesterone injected group were higher than other groups. Blastocysts cell number in superovulated (128.62 ± 1.3) and superovulated-progesterone injected group (126.88 ± 1.6) was statistically significant difference with control ($P<0.001$). The progesterone injection without ovarian induction could improve embryo survival and implantation rate but after superovulation it could not improve its negative effects on the implantation rate.

P4 (Poster presentation 4):
Transfer of cryopreserved-thawed embryos in a cycle using exogenous steroids with or without prior gonadotropihin-releasing hormone agonist

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An important factor for implantation in frozen-thawed embryo transfer (FET) is exact synchronization between endometrial maturation and embryo development. The aim of this study was to investigate the outcome of frozen-thawed embryo transfer in exogenous estrogen plus progesterone without GnRH agonist and with GnRH agonist cycles for endometrial preparation in women with regular menstrual cycles. This study was designed as a prospective randomized clinical trial. In total, 60 patients were randomly divided into two treatment groups. In both groups, Estradiol Valerate was taken orally at 2mg/day from day 1 to day 4, at 4 mg/day from day 5 to day 9, and at 6mg/day from day 10 onwards up to the day of pregnancy test. In day 13 of cycle, an ultrasound examination was performed. If the endometrial thickness was more than 8mm, progesterone was administered, i.m, at a dose of 100mg/day. Group A (n=30) commenced steroid supplementation without prior pituitary desensitization; whereas group B (n=30) had pituitary suppression prior to steroid hormone administration from day 21 of the menstrual cycle. The woman's age, duration and etiology of infertility, number of embryos transferred, and the score of embryo transferred showed no significant differences between the groups The implantation rate, chemical and clinical pregnancy rates were 1.6%, 10% and 6.6% in the group A, and 3%, 13.3% and 10% in the group B. There was no significant difference between both groups in implantation and pregnancy rates. Endometrial preparation for FET based exclusively

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on steroid administration appeared to be as effective as the protocol involving preliminary desensitization with a GnRH agonist.

P5 (Poster presentation 5):
Prevalence of urogenital infection with *Chlamydia* trachomatis in asymptomatic men from Tehran, Iran

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Chlamydia trachomatis is a common curable, bacterial sexually transmitted disease. The world health organization (WHO) estimated that 90million cases occur annually on global basis. The epidemic of Chlamydia trachomatis (CT) has been well documented in adolescent females, but information is limited regarding its prevalence among a healthy population of sexually active adolescent males. The main purpose of this study is to determine the prevalence of urogenital infection with Chlamydia trachomatis in asymptomatic men attending reference laboratory of Tehran Bu-Ali hospital from Dec 2005 to may 2006.This was a cross-sectional study on 140 asymptomatic 15-49 years old men attending to reference laboratory of Bu-Ali hospital. The method of sampling was random sampling and the samples were F.C.U (Participants had not voided for the least 2 hours). Mean while, Participants completed a questionnaire containing personal identifications such as age, marriage status, education, job and etc. Demographic data and PCR results analyzed by SPSS ver: 11. From 140 urine samples only 1(0.7%) was positive for *Chlamydia* trachomatis by PCR (%95 CI: %0-%2.07). Based on prior multiple and valid studies, screening women populations with 3.1–10 % *Chlamydia* trachomatis prevalence or screening of women and men in populations with at least 6% prevalence is cost effective. Based on this study screening for *Chlamydia* trachomatis by PCR in Iranian young asymptomatic men is not recommended. However, for evaluating the true prevalence rate wide studies with large sample sizes are needed and PCR. It seems that for epidemiologic studies serology is more cost effective than PCR is low prevalence populations. PCR seems to be more useful in symptomatic patients, and for epidemiological studies other cost effective tests are preferred.

P6 (Poster presentation 6):
The knowledge of the pregnancy induced hypertension in Iranian pregnant women and the effect of a simple Educational interventional measure

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Pre-eclampsia which constitutes 14 percent of maternal mortality in developing countries is particularly sinister because in its early stages a woman may be totally unaware of its presence. Taking a blood pressure measurement and testing the urine for proteinuria may reveal its presence. Through educating the at-risk population, a higher level of knowledge and awareness of the condition for mothers can be achieved. This is a key factor in early diagnosis and control of pre-eclampsia. In this survey the level of knowledge and attitude concerning pre-eclampsia is studied before and

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after a special program aimed at educating mothers. This study was done on 70 pregnant women using a reliable and valid self-administered questionnaire. The data were analyzed using the Statistical Package of Social Science (SPSS Inc., Chicago, IL). The score of knowledge of risk factors, signs and symptoms before and after education was 44+20.3 vs. 76.4+16.5. The score of attitude changed from 57.5+19.7 before the education to 92.3+8.4 after it. Fifty two percent of mothers showed low and very low level of knowledge. Knowledge showed no statistically significant correlation with the frequency of pregnancy, number of delivery or age. The results indicated that the level of knowledge and attitude of pregnant women increased significantly after education. Considering the low level of knowledge in more than 50 percent of women before education, it is established that there is a necessity to have special education with the relevant curriculum for those specific at-risk population on the one hand and the health system workers on the other.

P7 (Poster presentation 7):
Hormonal dynamic underlying hot flushes in perimenopause

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Hot flushes are one of the most common symptoms of menopause transition. However, they start earlier in late reproductive years and continue a few years after menopause. Hormonal changes following relative lack of sufficient follicles in the ovaries are believed to be the main pathology behind this symptom. In this study, 19 young control and 44 mid age women have recorded their hot flushes frequency and severity for 2 or more cycles and their FSH, oestradiol, progesterone and inhibins were measured in one cycle plus first 8 days of the second cycle. Results of the study suggested that sever hot flushes are associated with significantly lower oestradiol (Mean ratio: 0.73, 95% CI: 0.59- 0.89, P<0.005), higher FSH (Mean ratio: 2.2, 95% CI: 1.77- 2.71, P<0.005) and lower inhibin-A (Mean ratio: 0.7, 95% CI: 0.55- 0.94, P<0.05). Progesterone and inhibin-B were also decreased in the cases with severe hot flushes, however, the difference were not significant. It was also shown that the degree of deviation of oestradiol from its mean during the cycle could be correlated with the magnitude of hot flush score, particularly if is not opposed by similar degree of deviation of progesterone. This finding was shown using the coefficient of variation (CV) of the hormones in the cycles. Comparing the pattern of hot flushes with the hormonal data in individuals indicated that there is no clear and distinct relation between any of the aforementioned hormones and the pattern of hot flushes. However, oestradiol level more than any other hormone could be correlated with hot flushes pattern. Inhibins especially inhibin-A were also observed that can have an association with hot flushes pattern. Rise in inhibins levels especially inhibin-A could possibly boost the anti hot flush effect of oestradiol rise if happens simultaneously.

P8 (Poster presentation 8):
Isolation, Screening and Transfer of Cellulase Gene from *Bacillus subtilis* to Lysine Producing Mutants of *Brevibacterium flavum*

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Microbial enzymes (e.g. cellulases) are very important in industry for digestion and removal of undesired polymers. Bacillus subtilis has been reported to secrete considerable quantities of cellulases and can successfully digest cellulosic materials into glucose while Brevibacterium flavum is industrial producer of lysine but it lacks detectable extracellular cellulase activity. B. flavum, used for lysine production in this study, was grown on nutrient agar medium at 30 ^{o}C for 24 hours. The mutagen, N-nitroso-N-ethylurea (ENU) was used for hyper-expression of lysine. Cells were exposed to 35 m*M* ENU for 5-30 minutes. After washing the bacterial pellet with sodium citrate buffer and growing on seed culture medium, lysine production was enhanced up to 125 g/L in the culture exposed to the mutagen for 5 minutes as compared to 81 g/L, produced from wild type bacterial cells. Optimization of different growth parameters showed that 30 ^{o}C temperature, 7.0 pH, 2 g/L methionine and threonine each, 20 g/L glucose as sole substrate for bacterial growth, 2-12 g/L lactate as growth enhancer, 24 hours incubation time was optimum for bacterial growth. In the next step of study, Bacillus subtilis was grown on Lauria-bertani growth medium and 1.92 µg/µl DNA was isolated by using genomic DNA isolation kit. After RNase treatment, the amount of DNA obtained was 1.7 µg/µL. Gel documentation for time course experiment (15-180 minutes) showed that *Bam*H1 digestion of DNA for 180 minutes was the most effective. Rapid estimation of DNA concentration with ethidium bromide dot quantification procedure yielded 150.25 ng/µl DNA. Ligation into *Bam*HI digested pUC19 vector was carried out with rapid DNA ligation and transformation kit. After DNA transformation in E. coli by heat shock method, DNA libraries were screened on agar plates with 12.48 ml/L ampicillin for cellulase genes followed by transformation in B. flavum mutants, which is future work of this study.

P9(Poster presentation 9): Differentiation of embryonic stem cell derived neurons to cholinergic neurons *in vitro*; and *in vivo* after transplantation in the rat model of Alzheimer's disease

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Cholinergic system is one of the important systems of mammalian central nervous system. Cholinergic neurons distributed in brain and spinal cord and contributed to principal functions like: consciousness, learning and memory, and motor control. Pluripotent embryonic stem cells can give rise to neuroectodermal derivatives in culture. This potential could be harnessed to generate neurons and glia for cell-replacement therapies in the brain and spinal cord neurodegenerative diseases. The aim of this study was to evaluate the effect of sonic hedgehog peptide (Shh) and Retinoic Acid (RA) and some other neural differentiation factors on differentiation of neural progenitor cells (NPCs) produced by lineage selection method from mouse embryonic stem cells to cholinergic neurons. Also these produced neural cells were transplanted in brain of NBM (Nucleus Basalis Magnocellularis) lesioned rats and the differentiation potential and therapeutic effects of

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grafted cells in improvement of spatial memory function were investigated. Royan B1, mouse embryonic stem cells derived from C57BL/6 strain was used to produce aggregates. Aggregates were cultured in serum free medium to produce neural progenitor cells (NPCs), and then NPC expansion was achieved by treatment with epidermal growth factor (EGF) and fibroblast growth factor (FGF2) in DMEM/F12 medium. Following withdrawal of EGF and FGF2, the cells were further cultured in presence or absence of Shh and RA and other factors for 5-8 days in a low serum containing medium. These cells were then prepared for transplantation or cultured for another 7 days in Neurobasal medium containing 10% serum and B27 factor and N2 supplement for differentiation toward neurons. Relative number of neurons and cholinergic neurons were revealed by immunocytochemical staining procedures using antibodies against MAP2, β-Tubulin3 and ChAT. RTPCR analyses were also performed to evaluate the expression of specific neuronal markers in different cultural steps.Bromodeoxyuridine (Brdu) labeled neural cells and ES cells were grafted after bilateral Nucleus Basalis Magnocellularis (NBM) lesioning by Ibotenic Acid (a specific cholinergic neuronal toxin) in male Wistar rats. After surgery, all animals were given antibiotics for 1 week and a daily immunosuppressant (Cyclosporine A, 10 mg/kg, i.p) until sacrifice. Spatial memory tests (Morris water maze test) were performed in neural cell grafted, ES cell grafted, normal, lesioned and sham lesioned groups. After memory tests the brain of rats were fixed by perfusion of 4% paraformaldehyde and were sectioned then floating sections were stained by immunofluoroscent techniques using antibodies against ChAT, B-Tubulin3, GFAP, and also for Brdu and mouse specific neural cell adhesion marker (NCAM) to determine the grafted cells from host cells.Data obtained show that around 70% of cells were MAP2 or B-tubulin3 positive. We found ChAT immunoreactivity in cultured cells in both treated and control groups but the percentage of cholinergic neurons was significantly $P<0.05$ greater in RA and Shh and LIF treated cultures than non treated groups. Our data from grafts indicated that transplanted cells were differentiated to neurons and glial cells and also cholinergic neurons. Morris water maze tests showed the significant decrease in time latency to finding the plate in neural cell grafted group compares with lesioned and ES cell transplanted groups. This study Indicated that some of the neurons produced by lineage selection method were cholinergic, and the percentage of cholinergic neurons increased after treatment by Shh, LIF and RA. These cells could be transplanted and differentiated *in vivo* to neural cells. Behavioral studies revealed that the transplanted cells could replace as host neurons and attenuated Ibotenic acid-induced neurological symptoms.

P10 (Poster presentation 10):
The serum prepared from the rat peripheral blood significantly improves the viability and proliferation indexes of the rat mesenchymal stem cells *in vitro*

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All current protocols for *in vitro* culture of mesenchymal stem cells (MSCs) include fetal bovine serum (FBS) as nutritional supplement that is an undesirable additive since carries the risk of transmitting viral and prion

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diseases. For clinical application, however, contact of MSCs with FBS should be minimized. To overcome these problems, one strategy would be replacing FBS with autologous serum. Studies related to this subject are very rare and rather controversial. Therefore, in the present experiment we designed to study the viability and proliferation of rat MSCs during isolation and expansion period using the medium containing the serum prepared from their own peripheral blood versus currently-used FBS. For this purpose, rat bone marrow cells were cultivated in a DMEM medium containing either 15% rat peripheral blood-derived serum (PBDS) or commercially-purchased FBS for three successive subcultures during which the morphology, viability and growth kinetic of the cells were examined. Passaged-3 cells from either group were easily differentiated into osteoblastic, chondrocytic and adipocytic cell lineages, which was indicative of their MSCs nature. In present study, the viability of the cells was studied by MTT test and the growth kinetic were investigated by colony forming assays , growth curve study as well as the calculation of population doubling number (PDN). Each experiment was replicated several times and the data from each evaluation was statistically analyzed and compared to each other.passaged-3 MSCs from FCS group seemed to be somewhat shorter, broader and clear interface than the cells from PBDS group. Colony forming assay showed that PBDS-cultured cells were significantly more colonogenic than the cells cultured in FCS-contained medium (for instance 85±2.705 versus 62±6.8479 colon per 100 cells for passage 3) in all subcultures. MTT results indicated that all cell cultures in the PBDS medium have significantly higher absorption rate than of those in FBS medium. According to the growth curve study we found that the cells cultured in the PBDS medium had a similar lag phase as the cells cultivated in FBS medium but the two cultures showed their growth curves log phase that was sharper and longer in PBDS than FBS group and reaching to platue has been delayed compared to that of FBS-grown cells. Our results also indicated that the cumulative PDN for the cells of PBDS group was approximately twice of the cell from FBS group and this difference was statistically significant ($p < 0.004$). Our results indicated that the use of the rat own serum for isolation and expansion of their MSCs could significantly improve the viability and proliferation index of the cells while maintaining their differentiation potential during the cultivation period. Autologous serum did not carry the risk of transmitting viral and prion diseases and can be used without of any concern for isolating and expanding of the bone marrow-derived MSCs that are intended to be used for transplantation purpose.

P11 (Poster presentation 11):
The effect of pregnant mouse serum on dendritic cells in inducing T cells non-specific and specific responses

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Tolerance of the semi-allogenic fetal graft by the maternal immune is a medical enigma that has stimulated researches for a half of century several hypotheses have been proposed for tolerance of the mother to the fetus. The successful pregnancy is proposed and proved by many scientists to be Th2 dominant phenomena. This hypothesis is proved in most aspects of feto-maternal interface, but systemic effect

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of pregnancy on immune system, are controversial. Dendritic cells (DCs) are the most potent activators of naïve T lymphocytes capable of tolerance induction as well as immunity. These cells can influence Th cells differentiation, by inducing Th1 or Th2 responses as well. Therefore DCs are one of the probable candidates which mediate immune regulation during pregnancy. The aim of this study was to determine if pregnant mouse serum has any effect on DCs' functional capacity to stimulate non-specific and specific proliferative response of T lymphocytes. Mid-gestational sera were obtained from allogenic pregnant Balb/c mice (Balb/c × C57BL/6) on days 9-11 of gestation. DCs were purified from Balb/c mice spleens through a three step method including collagenase digestion of spleen tissues, selection of low-density cells by Nycodenz density gradient medium and plastic adherence. The purity of DCs was determined by flowcytometry, using anti CD11c Ab. The T cells were isolated from C57BL/6 mouse lymph node through Nylon wool method and their purity was determined using anti-mouse CD3 antibody. DCs isolated from spleen of pregnant or non-pregnant Balb/c mice were co-cultured with allogenic C57BL/6 T lymphocytes prepared by nylon wool method from brachial lymph nodes. Some cultures of non - pregnant female DCs were treated by 2.5% serum obtained from pregnant mice at early, middle or late gestational periods and were used in the same mixed lymphocyte reaction (MLR) settings. Cell proliferation was measured by ^{3}H thymidin incorporation and cytokine production was measured in supernatants of MLR cultures using ELISA method. Effect of pregnant mouse serum on expression of DC surface markers was evaluated by flowcytometry. In order to determine the effect of pregnant mouse serum on DCs in inducing T specific proliferative responses, some cultures of non-pregnant female DCs were pulsed with Conalbumin as a foreign antigen during overnight culture. In some cultures pregnant mouse sera were added at 2.5% final concentration and some cultures were treated with non-pregnant sera at the same concentration. Antigen pulsed DCs were injected in to mice front footpads. Draining lymph node cells of immunized mice were cultured in presence of Conalbumin and after 5 days and their proliferation was measured by ^{3}H-thymidin incorporation method. No significant difference was found between stimulatory potential of splenic DCs from pregnant and non–pregnant mice in induction of allogenic T cell proliferative response. Moreover, serum of early or late pregnancy did not have any effect on DCs function in comparison with non-pregnant mouse serum, while mid–pregnancy serum significantly inhibited allostimulatory activity of DCs. IFNγ production in co-culture of DCs treated with pregnant mouse serum was significantly lower than that of control group, however, no significant difference in IL-10 production was observed. Treatment of DCs with pregnant mouse serum did not influence the percentage of cells expressing MHC-II, CD86, CD8α or CD11b. However a marked reduction of the mean fluorescence intensity of MHC-II was observed. The results of pregnant mouse serum effect on DCs in inducing T cells specific proliferative responses showed that normal serum-treated, antigen-pulsed DCs induced a strong proliferative response of T cells however; treatment of DCs with pregnant mouse serum markedly blocked their ability to induce antigen-specific lymphocyte proliferation. We demonstrated for the first time that serum obtained from pregnant mice at mid gestational period is capable of suppressing the allogenic and Ag

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specific activity of DCs in inducing T cell prliferative responses. The lower concentration of IFNγ in supernatant of allogenic MLR when DCs were treated with mid pregnancy serum indicate that such treatment favors induction of TH2 immunity which is advantageous for successful pregnancy. It seems that there is a systemic state of immunosuppression affecting DCs function during mid gestation and it is micro environmental instruction that dictates how DCs exert their functions. The exact mechanism by which pregnant mouse serum inhibits allogenic and also Ag specific capacity of DCs in inducing T cell Proliferative responses is unclear, but it is probable that immunosuppressive molecules such as pregnancy-specific glycoprotein a (PSG1a), prostaglandin E2 and soluble HLA-G which present in higher concentration during pregnancy are responsible for this phenomenon.

P12 (Poster presentation 12):
Cell cycle stage analysis of sheep granulose cells

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Somatic cell nuclear transfer has a low success rate. The stage of the donor cell cycle is an important factor that may affect the successful development of the nuclear transfer embryo. The use of nuclei at G0/G1 phase improves the development of reconstituted embryos. In the present study, the synchronizing effect on granulose cells which are frequently and successfully used as donor cells in cloning experiments has been investigated. Sheep ovarian were washed in PBS after being transferred to the laboratory. Granulose cells were obtained by aspirating the ovarian follicles and were cultured in DMEM medium until forming a confluent monolayer, and harvested by trypsinization. After reaching 70-80% confluency, the medium in group was changed with DMEM containing 0.5mM mimosine and left for 24 h, the medium in group was replaced with DMEM containing 0.5 g/ml Nocodazole and left for 20h. In group III, IV and V the cells were starved with DMEM containing 0.5% serum for 24, 48 and 72h. The DNA synthesis, cell cycle stage and apoptosis of the cultured cells in all groups were then examined by immunocytochemical detection of incorporated 5-bromo-2-deoxyuridine (Brdu), flow cytometry and apoptosis detection kit respectively. Treatment of cells with mimosine, nocodazole and serum starvation for 48 and 72 h, resulted in proliferation arrest *in vitro*($p < 0.05$). Serum starvation for 24h did not significantly decrease DNA synthesis. Analysis of cell cycle distribution by flow cytometry showed 75.8, 6.04 and 18.08% of confluent cells at G0/G1, S and G2/M respectively. Serum starvation for 24, 48 and 72h arrested 78.05, 83.5 and 91% cells at G0/G1 phase. Treatment with mimosine and Nocodazole resulted 85.2 and 79% cell arrest. Under normal culture conditions 4.7% of cells underwent apoptosis. Serum starvation for 24, 48 and 72h caused apoptosis of 5.1, 5.6 and 6.1% of the cells (for 72h $p < 0.05$). Mimosine (4.8) and Nocodazole (4.9) treatment did not increase the number of apoptotic cells significantly. Both serum starvation for 48 or 72 h and mimosine treatment effectively synchronized sheep granulosa cells in G0/G1 phase and cell death in these two groups was very similar to the control group. Nocodazole treatment did not increase G0/G1 cells significantly. Serum

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starvation for 3 days significantly promoted apoptosis in granulose cells.

P13 (Poster presentation 13): Biological effects and potential hazards of microwaves used in cellular phone system on infertility

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Although recent researches approved that EM waves cause harm to CNS and bone marrow systems, they are not studied to have health effects on other systems of human body. Nevertheless, possible health effect of EM waves on reproductive system in males and females is a question as this system has also dividing cells (like CNS and bone marrow). In this case possible effects of E-M waves on infertility are a matter of concern. This research is done to investigate infertile couples about possession and usage methods of mobile handsets and also to discuss the role of the proximity of housing/occupation to BTSs of GSM (900, 1800) mobile phone network as a means of possible cellular phone exposure and so having possible influence on infertility. The research has been done by questionnaires and as cross-sectional. Collected data then was analyzed using SPSS software. The results of the study show that these variables have correlation with infertility:

1. Duration which these patients use cellular phone for speech
2. Place of holding cellular phone regarding body
3. Model of cellular phone
4. Proximity of the handset regarding body

At the end, based on the results of this research a device was invented and registered as a patent to measure the power of EM waves in the environment and to alarm when its holder is exposed to the dangerous waves for infertility**.**

P14(Posterpresentation14): Raloxifene effects on cultured human endometrial stromal cells

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Raloxifene is a selective estrogen receptor modulator (SERMs) that is used to prevent osteoporosis in postmenopausal women. SERMs are capable of inducing specific changes in the estrogen receptor, and they may mediate specific pharmacologic activity through their unique agonist or antagonistic properties. Its apparent rule is lack of proliferative effects on endometrial tissue. Regarding to the importance of stromal cells in the pathogenesis of endometriosis and adenomyosis، the Aim of the present study is to determine the effects of Raloxifene on growth rate and number of human endometrial stromal cells *in vitro*. In this experimental *in vitro* study, endometrial biopsies (n=8) prepared from women (age=25-40) year without malignancy, polyps and hormone therapy which informed consent. Tissues washing with hank's solution are done; samples are cut into very tiny fragments. After enzymatic digestion, filtration and layering with Ficol's solution, stromal cells were separated, counted and were cultured. Stromal cells suspension were divided to four groups: control which received DMEM-F_{12} supplemented with FBS, Antibiotics and three case groups cultured in DMEM–F_{12} supplemented whit FBS, Antibiotics and Raloxifene

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(0.1, 1, 10) μm. Every 3 days cell cultures were observed by invert microscope (Motic–AE 31) and the medium were exchanged. After 15 days, stromal cells harvested and counted. The occupied area of culture plate with stromal cells determined by Motic software and compared with control groups. Morphological changes of stromal cells on day 15 of cultures between case and control groups are determined by two histologists under invert microscope through two methods. Data were analyzed by Friedman and paired sample tests. Raloxifene Doses (0.1, 1, 10 μm) significantly decrease the growth rate and the number of cultured stromal cells ($p<0.05$). Showed that morphological changes were significant between (0.1μm, 10μm), (1μm, 10μm), (0.1μm, control) و (1μm, control) و(10μm, control)groups ($p < 0.05$) but the changes between (0.1μm, 1μm) were not significant. Raloxifene (0.1, 1, 10 μm) has inhibitory effects on number of cultured endometrial stromal cells and has significant effects on morphology of endometrial stromal cells.

P15 (Poster presentation 15): Fetal growth and development from the viewpoint of Quran

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Quran's miracles have been noticed by Islamic scientists and thinkers. One of Quran's miracles is scientific points that nowadays they might be explained by science. Fetal formation is one of the interesting scientific views of Quran. The aim of this article is evaluation of 6th verse of *Zomer Sura* (6/39). In this verse it has been pointed that fetal growth and development in mother's uterus is doing in to three dark spaces which are named abdomen, uterus and fetal membranes. This article tries to compare new scientific information with Islamic scientist ideas and also presents the common scientific views between them.

P16 (Poster presentation 16): The correlation of cesarean section and subsequent subfertility

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Cesarean section rates have risen rapidly throughout the developed world during the last 20 years and now stand at approximately one birth in five. Many factors have contributed to this rise, including maternal choice, increased maternal age and more liberal use of the procedure for women with previous CS, breech and multiple pregnancies. Concerns have been expressed about the financial costs of the operation and the consequences for the women involved. Whilst recent reviews have reported some conventionally held beliefs about the potential benefits of contradictory and could as easily be used to make a cause for increasing the CS rate. One of the issues causing widespread concern is the effect of CS on future fertility. The aim of this study was to determine the correlation between cesarean section and subfertility.

This systematic review assessed more of 16 studies from books, articles and different sites of internet about the correlation between cesarean section and subfertility in 1998-2006.Heminki et al. (1998) reported that women who have their first baby by CS tend to have fewer children and only 47% if those delivering by Cs went on to have a

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subsequent pregnancy over a 5-year period compared with 57% of those having an instrumental delivery and 62% of those with a spontaneous vaginal delivery .Lietch et al. (1999) found that 50% of women having a Cs didn't return with another pregnancy. Jolly et al. (2002) showed that 42% of women who delivered by Cs, had no further children after 5 years compared 29% of those with a spontaneous vaginal delivery. Morphy et al.(2003) also demonstrated an association between Cs and prolonged time to conception. 14.4 % of those having a CS taking more than a year to conceive compared to 8.5% of those not having a CS. Epidemiological studies have confirmed an association between Cs and infertility. What has been less widely studied is the nature of the link. As yet it is unclear whether reproductive potential is compromised by the effect of pelvic surgery, or whether women are deliberately limiting their fertility following CS. Other findings will explain in full text of paper.
All of the Epidemiological studies about the association between Cs and subfertility, reported the negative association between Cs and future fertility .What are needed now are qualitative studies to determine the role and nature of such correlation. Reliable evidence on long term consequences of cesarean section is essential if women to be offered informed choice with regards to mode of delivery.

P17 (Poster presentation 17):
Effects of anabolic-androgenic steroids on the testis in adult male rats

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Anabolic-Androgenic Steroids (AAS) Compounds are used by athletes, prepubescent and adolescents for improving athletic ability muscle mass or body style. Therefore, administration of these compounds is increased significantly. Many of the athletes who use AAS believe that the side effects on the male reproductive function have been reported but little in known about how these compounds effect sexual behavior and fine structure of the male gamete and supportive tissue of testis of long term administration of AAS compounds on the fine structure of the testis, as well as body weight, testis and epididymis weight, and also sperm parameters. Seventy two Sprague-Dawly male rats (3 month) were randomly classified into two experimental (N = 38), two vehicle (N = 24) and one control (N = 12) groups. Experimental rats were intramuscularly treated with 3mg/kg and 10 mg/Kg Nandrolon Decanoate and vehicle groups with same dose of peanut oil weekly for 14 weeks (intramuscularly). Control group was kept under standard condition. After sacrificing body weight, testis and epididymis weight and also sperm parameter were assessed. To investigate fine structure, both testis of five rats from each group were prepared, using routine tissue processing for TEM. There were no significant differences between body weight treatment and other groups. The experimental groups showed significant differences ($P<0.01$) in testis and epididymis weight with other groups. Ultrastructuraly, interstitial space of the Experimental groups showed a considerable decrease in the cell counts. Degenerative changes were revealed in leydig cells. The present study showed thickness dye to sclerosis and dentate of the lamina properia. The thicker lamina properia accompany with a significant reduction in tubule diameter.

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Cytoplamsic vacuolization of sertoli cells in the most of tubules were observed in experimental groups. Several Apoptotic germ cells were found in the seminiferous epithelium of the experimental groups. Significant decreases of sperm count and motility percent, as well as percent of abnormal shape of sperm were observed in experimental groups. The results of this study show that AAS compounds affects the fertility parameters and causes testis and epididymis atrophy and changes of the testis ultrastructure. This study is a sing of alarm for subjects that freely consume steroids in order to decrease the time required for increasing muscle mass using only exercise routines.

P18 (Poster presentation 18):
Clonal characterization, isolation and differentiation of mesothelial stem/progenitor cells of the peritoneum

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Mesothelial progenitor cells have been reported to reside in either the monolayer of mesothelium, submesothelium or within the peritoneal cavity as free floating cells. A putative plasticity has been reported for these cells as, an epithelial to mesenchymal transition as well as the transformation into myofibroblasts and smooth muscle has been reviewed in previous works. In order to investigate the differentiation of these cells**,** we conducted an in vivo tracking of transplanted mesothelial cells after isolation and characterization of mesothelial cell clones within the peritoneal dialysis liquid from early stage non peritonitis patients for mesothelial, tumor and hematopoietic cell lineage markers, having in hand the common developmental background of hematopoeitic and mesothelial and mesenchymal cell lineages. On days 14, 30 and 60 post transplantation to immunodeficient mice, brain, heart, skeletal muscle and lung tissues were screened by FISH (Fluorescent In Situ Hybridization) directed to the Y chromosome by flow cytometry, fluorescent microscopy and PCR, demonstrated the existence and morphology alteration of the donor cells in various organs of the recipient mice, notably in the skeletal muscle and lung and less in the heart and brain. Immunostaining of recovered cells from the nervous recipient tissues suggests differentiation of mesothelial cells in the new microenvironment. Secondly we defined specific media for the differentiation of the progenitor cells of the peritoneum towards neurons, skeletal muscle cells and osteoblasts. We isolated and analyzed mesothelial clonal population that expressed CD34, CD31, HBME-1, markers but insignificantly express WT-1, CD90. Mesenchymal cells which became dominant through continuous culture expressed mesenchymal stem cell markers such as: CD44 and cells were positive for alkaline phosphatase activity. A recent report suggests that mesothelial stem cells express markers of the mesothelial itself. The culture of mesothelial cells, harboring these populations, shows an incredible differentiation capacity in knockout serum cultures with specific growth factors. We were able to specify differentiation to neurons, osteoblasts and skeletal muscles by morphology and histology studies, immunostaining

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and RT-PCR. Differentiation towards neurons was induced by RA and NGF treatment in knockout ES certified FBS and DMEM –F12 media. The resultant neurons were testified for neurofilament 200, Nestin, Tubulin B which confirmed the neuronal nature of these cells. Differentiation towards skeletal muscle was induced by TGF-B and Dexamethasone .Muscular identity of the cells was obvious by the expression of troponin1 skeletal muscle, heavy chain myosin. Differentiation towards osteoblasts was induced by B-glycerophosphate, TGF-B and Dexamethasone. Osteocalcin, oil red and ALP was also positive. Our data completely violates the previously assumed plasticity of mesothelial progenitor cells and lead us to the definition of a new source of adult stem cells.

P19 (Poster presentation 19):
The effect of administration of metformin on lipid profile changes and insulin resistance in patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOs) is one of the most common metabolisms and endocrine disorders among women. The aim of the present study was to evaluate the effects of metformin on lipid profile changes and insulin resistance in patients affected by PCO syndrome. In this randomized study, 200 women (18- 35years) with PCOS were selected. Diagnostic criteria were based on the diagnostic criteria of PCO syndrome in Rotterdam meeting in 2003. The sample of fasting peripheral blood was taken from all patients in order to test cholesterol, LDL, HDL, TG, FBS and Insulin. The patients were divided randomly into two groups. In case group (n=100), metformin was prescribed three times a day (1500 mg daily) and in control group (n=100), placebo was administered in the same way. After three months, blood sample was taken again in order to test the variance of the above mentioned parameters in order to compare with these amounts before test. Also, Body mass index (BMI) was compared before and after treatment between two groups. BMI was 28.81±3.18 and 29.49 ± 4.7 Kg/m^2 before treatment in case and control groups respectively. This ratio changed to 28.45 ± 2.8 and 29.29 ± 4.8 Kg/m^2 after treatment in case and control groups respectively. ($P > 0.05$) FSH/insulin ratio was 4.67±0.9 and 5.03±1.3 in case and control group respectively, which changed after treatment to 6.07 ± 1.4 and 5.05 ±1.3 and this difference was significant in case group (P-value=0.0001),but it was no difference in control group. In case group, HDL level was increased after treatment from 26.65±9.9 to 33.19± 9.9 mmol/L (P=0.0001), and Triglyceride level was decreased after treatment from 208.96±58.9 to 191.54±55.4 mmol/L (P =0.004); whereas there was no change in control group. LDL and cholesterol levels did not change in both groups. Treatment with metformin will cause the decrease in insulin resistance and lipid profile changes. Therefore, it can be useful for preventing the long term complications in PCO patients.

P20 (Poster presentation 20):
The role of sperm parameters on the outcome of intracytoplasmic Sperm injection

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Because one spermatozoon is directly injected into the oocyte during Intracytoplasmic Sperm Injection (ICSI), spontaneous abortion may be opportunity for the elimination of genetically abnormal embryos. In addition, the severity of spermatogenic impairment is positively correlated with chromosomal abnormalities. So, Sperm abnormalities maybe correlate with clinical pregnancy. The aim of this study was to investigate the role of sperm parameters on ICSI outcome. This nested case-control study was conducted in Isfahan Fertility and Infertility Center (Isfahan, Iran), a private IVF center. A total of 523 couples undergoing ICSI cycles between January and September 2006 included in study. Beside routine workup, sperm samples obtained from male partner and analyzed, ICSI performed and female partner followed to determine if pregnancy achieved. Couples grouped in pregnancy positive and pregnancy negative groups, subsequently. This leaded to 106 pregnancy positives as cases, and 417 pregnancy negatives as controls. Semen volume, sperm concentration, percentage of sperms with abnormal head, neck and tail, percentage of sperms with progressive, sluggish and no motility, sperm agglutination and leukocytospermia were compared in two groups. Statistical analysis performed using Student's T-test and Pearson $\chi 2$ test. A P level <0.05 was considered significant and minimal desired power was considered 0.80. Sperm concentration (P=0.043), percentage of sperms with abnormal head (P=0.019), leukocytospermia (P=0.026) and sperm agglutination (P=0.006) was significantly different in pregnancy positives and pregnancy negatives. There was no significant difference in percentage of sperms with abnormal tail (P=0.844, Power=0.885) between two groups. About other parameters including semen volume (P=0.230, Power=0.703), percentage of sperms with abnormal neck (P=0.331, Power=0.525), with progressive motility (P=0.134, Power=0.501), with sluggish motility (P=0.412, Power=0.558) and with no motility (P=0.122, Power=0.500), there was "no evidence of effect" rather than "evidence of no effect" on ICSI outcome. We obtained in contrast to majority of previous researches that some sperm parameters correlate with ICSI outcome. So, these parameters may point to future failure of ICSI. That is unclear if these correlations are causal, and further research is needed to investigate definite effects of these parameters on ICSI outcome.

P21 (Poster presentation21):
Study of CatSper gene expression in contusion model of mouse spinal cord injury

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Spinal cord injury (SCI) has a greater impact on sexual and reproductive function in men. Infertility is one's major problem associated with SCI .Sperm quality such as low sperm viability, low motility and increase sperm abnormal morphology are the elements involvement with infertility following SCI. CatSpers genes are specification channels in sperm and play an important role in sperm motility and hyper activation. In the previous studies demonstrated various abnormalities in spermatogenesis. The expression specificity in testis of the CatSper genes after SCI was not studied, so in this study our purpose was to reveal the changes of CatSpers

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genes which happen after contusion SCI. A total of the 75 adult NMRI mice were divided into 3 groups of SCI, sham and control. Following laminectomy, SCI group was induced using a 2 g weight device dropped from a distance of 2.5 cm onto exposed dura matter of the 9th thoracic vertebral. Sham group underwent laminectomy of T9 only. While, the mice of control group were not exposed to any type of injury or medication. The epididymal sperm parameters were studied after one day, one, two, four and six weeks after injury. One testis was removed to do morphological study and others testis for molecular study. Total RNA was extracted from testis of mouse in different time of post surgery (1st day, 2nd weeks, 4th weeks and 6th weeks). cDNA was synthesized from RNA template and it amplified in PCR reaction for both CatSper and β2m (internal control) genes. By gel electrophoresis was done by PCR product and analysis them by UVIDoc version 12.4 software (Semi quantitative PCR). The data were analyzed with SPSS soft ware. Our results showed that SCI affected spermatogenesis, sperm quality and quantity. The sperm count was reduced from the fourth week and it had significant difference comparing control and sham groups ($P< 0.05$). Normal morphology rate of experimental group showed significant reduction (37.8 ± 1.9) in the end week of study comparing to control (88.48 ± 2.6) and sham groups (86.76 ± 2.4). Viability and total motility rates of experimental group also had significant differences with control and sham groups ($P< 0.05$). Meanwhile significant differences were observed in experimental group comparing control and sham groups. Histological study revealed that some seminephrous tubules of the experimental mice were depleted of spermatozoa. In molecular study there was not change expression of CatSper genes in mouse testis of 1 day, 1, 2 weeks after surgery but in 28 and 42 day following surgery significant changes take place in gene expression. The present study suggests that the contusive spinal cord injury causes reduction in the all sperm parameters and has got deleterious effects on spermatogenesis and there were changes in expression of CatSper genes after 4 weeks post surgery in mouse.

P22 (Posterpresentation22): Numerical chromosomal abnormalities assay by electrofusion technique after maternal chemotherapy with yclophosphamide in preimplantation mouse embryos

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Cancer therapies increase the life-span of young patients significantly. However, most of the anticancer drugs such as Cyclophosphamide have been shown to be potentially damaging agents. Cyclophosphamide causes gene mutations, chromosomal breaks and rearrangements in somatic cells, and can also cause menstrual irregularity and infertility in female patients. Females that are sub fertile due to chemotherapy can receive assisted reproduction techniques such as *In vitro* Fertilization (IVF), but we must be sure of the embryo health by some means. Two different groups consist of female NMRI mice, 2-3 and 6-7 weeks of age were injected intraperitoneally with 75 mg cyclophosphamide /kg body weight. The control groups were injected with sterile water. Six weeks later, the mice were superovulated with PMSG and

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HCG. Oocytes were recovered, washed and fertilized with sperm obtained from non-treated male mice, and incubated for 3 days in 5% CO2 in air. The fertilization rate and embryo developments were considered. In the present study we compared two methods for visualization of blastomer's chromosomes to establish a safer and more accurate method to detect chromosomal abnormalities in the treated groups. In the first method the blastomers of the eight cells embryos were arrested in the metaphase stage by colcemide treatment. The second method was metaphase induction by the electrocution technique. Briefly, a mouse zygote without zona pellucida electrofused with the blastomer separated from mouse embryo (embryo which maternally treated by chemotherapy agent) in interphase stage and then the chromosomes were evaluated. Cyclophosphamide in both experimental groups, reduced the oocyte fertilization rate and in the 6-7 weeks group fertilization rate was significantly lower compared with control group ($p<0.01$). Also there was reduction in embryo development in both groups ($p<0.05$). Aneuploidy increased in the treatment groups compared with control groups, which in the older group was significant ($p<0.001$). Success rate of heterokaryons formation with analyzable chromosomes was 68.5% in the electro-fusion method and 30.1% in the colcemide method. Results show that those patients who received treatment before puberty may have higher fertilization rate in the future. The electrofusion technique is very effective for performing chromosomal analysis. In regard to the defects in embryo cleavage, developments and the numerical aberrations in the chromosomes, we suggest pre-implantation genetic diagnosis especially with the electro-fusion technique in order to confirm chromosomal status of the embryos and increasing the pregnancy rate in these patients. It is necessary to increase the success rate in observing blastomer's chromosome before implementing it in the PGD procedure of the human embryo.

P23 (Poster presentation 23): Improved *ex vivo* expansion of isolated $CD34^+$ cells from human umbilical cord blood and their cotransplantation with or without mesenchymal stem cells in irradiated Balb/c mice

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Umbilical cord blood (UCB) contains a high number of primitive progenitor cells. This characteristic allows UCB to be as a source of hematopoietic progenitors for clinical transplantation. However the rate of UCB $CD34^+$ stem cells graft is low. Mesenchymal stem cells (MSC) have been implicated in playing an important role in hematopoietic stem cell engraftment. So that in this study we examined the effect of human MSC on engraftment of human umbilical cord blood (UCB)-derived $CD34^+$ cells in irradiated Balb/C mice. Human UCB $CD34^+$ cells were obtained from full-term normal deliveries by using an immunomagnetic separation technique and MSC were isolated by standard methodology from human bone marrow. The direct determination of the absolute count of $CD34^+$ was assayed by Flow cytometry. Isolated MSC characterized according by flow cytometric determination of

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cell-surface antigen CD166 and CD105 and their morphology. Viability test was performed by trypan blue staining. Isolated $CD34^+$ cells were cultured in Stemline Hematopoietic stem cell expansion medium supplemented with 100 ng/ml SCF, and 100 ng/ml TPO in 24-well plates and incubated at 37ºC in a fully humidified atmosphere with 5% CO_2 and maintained over three weeks and half the medium was exchanged twice a week. MSC were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). Irradiated (7 Gy) Balb/C mice were transplanted intravenously with 0.2 to 1.0×10^6 human UCB $CD34^+$ cells in the presence or absence of 0.25 to 1×10^6 human bone marrow-derived MSC. The mice in every group on day 11 after transplantation were killed and their spleen dissected. In every group colony assay were performed. For better study of colony in the spleen H&E staining was performed. For approving the presence of human stem cells in colony, UCB $CD34^+$ cells labeled with super paramagnetic iron oxide (SPIO) were transplanted. After establishing the presence of colonies in spleen, Prussian blue staining was performed. The average number of $CD34^+$ were obtained from every sample was 25 to 50×10^4 and MSC was 7×10^4 per samples. Flow cytometry assay showed that up to 90% purity of $CD34^+$ cells and 96% for MSC and trypan blue staining showed that percentage of viable cell was 100%**.** Also after three weeks the cell numbers were reach 1000-fold increasing of $CD34^+$ and after two month 500-fold increasing of MSC. Cotransplantation of low doses of UCB $CD34^+$ cells (0.2 and 0.3×10^6) and MSC (0.5 and 1×10^6) resulted in a significantly increasing in colony forming unit spleen, in comparison with engraftment of UCB $CD34^+$ stem cells without MSC after 11 days ($P < 0.01$). After Prussian blue staining Fe^{+2} granules was observed. This indicated these cells in the colony were UCB $CD34^+$ stem cells that were engrafted. In conclusion the results showed that usage two cytokines (SCF, TPO) was adequacy for expansion of UCB $CD34^+$ cells. Also cotransplantation of MSC with UCB $CD34^+$ cells; promote engraftment of UCB $CD34^+$ cells.

P24 (Poster presentation 24):
The comparative effects of follicular fluid and conventional culture medium on sperm histon quality: An Aniline blue staining study

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Fertilization outcomes depend on DNA of sperms and deficiency in the sperm chromatin could fail this phenomenon. Sperm preparation is very important in Assistant Reproductive Techniques (ART). One of the attempts in improving of ART results is preparing medium culture similar to the *in vivo* conditions. One Candidate for approaching this goal is follicular fluid. Therefore, this study was designed to evaluate the effects of incubation of follicular fluid in comparison with conventional media. Follicular fluid obtained from 36 healthy female donors and sperm obtained from 36 males who were referred to infertility centers in Shiraz. Sperm parameters of normal donor were in according with WHO criteria. Semen samples were washed with Ham's F-10, then sperms freely swimed up. Collected sperms were divided into control and experimental groups. The control group was incubated only in Ham's F-10 but the experimental group in Ham's F-10 plus 10% follicular fluid in different times. Smears were prepared for staining with

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aniline blue after 0 min, 20 min, 3h, and 24h. Sperms with high concentration of histons are stained dark blue and mature sperms are light blue. The percentage of immature sperms in the experimental group decreased after 24 hours of incubation in follicular fluid in comparison with sperms in the control group. The percentage of immature sperms did not show any significant difference during the 0min, 20min, and 3h in the both control and experimental groups after incubation. It can be concluded that follicular fluid composition can improve histons quality of the sperms and thereby fertilization outcomes. Further studies are necessary for improving the culture media.

P25 (Poster presentation 25):
Effects of L-acetyl-carnitine on motility and DNA quality of Testicular sperm in mouse

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The testicular sperms from tissue biopsy are frequently immotile. The purpose of our study was to assess the effect of short exposure of testicular samples to L-acetyl-carnitine on mouse sperm motility and DNA quality in culture medium**.** Testis of 30 mice were removed, testicular tissue samples obtained by open biopsy were placed into a Falcon tube containing 2 ml of medium (Hams F 10). The tissue was washed several times with Hams F 10 and then centrifuged at 2000 *rpm* 10 min in order to separate the red cells. Then tissues were put in 3 ml of Hams F10 and were minced by gentle crushing between two needles and agitated for 60 s on a vortex to separate the different cell types, supernatant was centrifuged for 10 min at 500 *rpm* for separate the other cells. Again the supernatant was centrifuged with 2000 *rpm* for 10 min. The pellets were divided into 2 groups. In control group sperms were treated in Hams F 10. In experimental group, testicular sperms were treated with Hams F10 plus L-acetyl-carnitine (1.76 mM).The motility was assessed after 0, 30 and 90 min of incubation. At the same times smears were prepared and stained with Acridine Orange too. Our results showed that the sperm motility of experimental group was higher than our control group after30, 90 minutes**.**-It has been demonstrated that DNA quality in our experimental group was much better than the control group after 30, 90 minutes. It can be concluded that L-acetyl- carnitine can improve motility and DNA quality of testicular sperm in mouse.

P26 (Poster presentation 26): Characterization of megakaryocyte progenitor cells differentiated from umbilical cord Blood CD133^{+} and CD133^{-} Cells .

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Umbilical cord blood (UCB) is a rich source of stem/progenitor cells. Low efficiency in adults and tardiness in platelet recovery are disadvantages of UCB transplantation & hematopoieitic reconstitution. Although, megakaryocyte (Mk) progenitor cells in UCB are more primitive. CD133 antigen is a five transmembrane (5–TM) glycoprotein selectively expressed in CD34bright subsets in bone marrow (BM), fetal BM and liver, cord blood and peripheral blood. Numerous studies have shown that UCB stem cells especially CD133^{+} subpopulation have

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more capacity for produce Mk progenitor cells and because these cells are more immature have more utilization in transplantation. Purpose of this study was determination of immunophenotyping of Mk progenitor cells differentiated from UCB $CD133^+$ $CD133^-$ cells under effect of cytokine cocktail including IL–3, IL–6, SCF and TPO *in vitro*. First $CD133^-$ and $CD133^+$ cells isolated respectively by negative and positive selection using MidiMACS separator and LS column in extremely strong magnetic field. Then by Stem Span media and identical cytokine profile contained IL–3 (20ng/ml), IL–6 (20ng/ml), SCF (25ng/ml) and TPO (25ng/ml) expanded for 7 days. Expression rate of Mk antigens was identified by CD34, CD41, CD61 and CD42b on day 0 and 7 of culture using flow cytometry. Cell viability was evaluated by 7–AAD (7-Amino Actinomycin D) in addition to Trypan blue. Results analyzed by Student's *t*-test and $p<0.05$ considered significantly. Data showed that mean purity of $CD133^+$ cells is approximately 70% and expression of megakaryocytic markers on $CD133^+$ cells always higher than $CD133^-$ cells. Maximum and minimum expression of megakaryocytic antigen related to CD61 and CD42b respectively. In summary, more than 90% of $CD133^+$ cells co-express CD34 antigen, have more ability to generate Mk colonies in vitro and by transferring expanded cells to BM transplantation recipients can increase repopulating capacity.

P27 (Poster presentation 27): Behavioral study of Huntington's diseased rats after mesenchymal stem cells transplantation

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As an inherited neurodegenerative disease, Huntington's disease causes a range of neuronal degeneration in neostriatum and neocortex. In its developed stages, it causes serious clinical defects in movements, recognition abilities and finally death. There is no effective therapeutic strategy for diminishing the motor disorders of Huntington's disease, meanwhile recent progresses in stem cell technology produced promising data for curing such diseases. In recent years, cellular transplantation has been considered as an effective therapeutic method for neurodegenerative diseases. In this paper, the effects of bone marrow derived mesenchymal stem cells were assessed in animal model of Huntington disease. After making ipsilateral lesion in striatum with Quinolinic acid, bone marrow derived mesenchymal stem cells, which had been isolated and purified from 4-6 weeks old rats, were transplanted into the damaged striatum. The efficiency of cellular transplantation for improvement of motor disorder was assessed by cylinder test and apomorphin induced rotation tests, during eight weeks after engraftment. Results show significant improvement ($p \leq 0.0001$) in motor disorders, examined by cylinder and apomorphin induced rotation tests. According to the results of this assay, cell therapy by means of bone marrow derived adult stem cells seems to be a promising method for treatment of neurodegenerative defects, especially Huntington's disease.

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P28 (Poster presentation 28): The effect of varicocele on chromatin condensation and DNA integrity of spermatozoa

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Varicocele that is dilation of pampiniform venous plexus above and around the testicle, occur in approximately 15% to 20% of general male population especially in adolescents. This anatomical abnormality is the most common causes of poor serum production and decreased serum quality. The mechanisms by which varicocele may lead to spermatogenic failure are no well recognized. Varicocele leads to venous stasis in testis with resulting heat stress hypoxia and accumulation of toxic metabolites. It is demonstrated that patients with varicocele possessed a significantly higher DNA fragmentation index (DFI) and sperms with nuclear anomalies than healthy fertile men. This may be correlated with an increase of reactive oxygen species in their semen samples. While there are some evidences that sperm DNA integrity is altered in varicoceles, the effects of this abnormality on sperm chromatin condensation is poorly understood. So, the aim of this study is the evaluation of sperm chromatin integrity in these patients.sixty men referring to andrology laboratory were categorized into 3 different groups. First a group of 20 infertile patients with varicocele, second a group of 20 infertile patients with abnormal semen parameters .finally a group of 20 fertile men who had normal spermatogram as control group. Semen analysis was performed according to WHO criteria in all of 3 groups. To evaluate sperm chromatin quality and DNA integrity, after fixation of sperm smears, Aniline blue (for detection of abnormal persistence of histones in sperm chromatine), Toluidine blue (for detection of DNA abnormalities), Chromomycin A_3 (for detection of protamine deficiency) and finally Acridine orange (for detection of insitu DNA denaturation) stainings were done in all of groups. After staining, the slides were analyzed by light and fluorescent microscopy and to determine the percentage of mature or immature sperms, 100 spermatozoa were counted in each slide. The data were analyzed by SPSS version 13software and are presented as mean± standard deviation. Statistical significance was set at $p< 0.05$. 60 cases have been evaluated. The mean of abnormal persistence of histone in sperm chromatin with Aniline blue test are 15.75±5.44, 40.60±14.71, 50.15±15.79 in control, infertile and varicocele groups respectively and the difference was significant ($p = 0.000$) There was a significant difference between three groups with regards to CMA_3 results (23.40±6.84, 41.45±10.07, 57.15±8.31 in control, infertile and varicocele groups respectively and pp = 0.000).The mean of DNA chromatin with Toluidine blue test are 16.7±8.55, 33.50±9.58, 60.85±15.61 in control, infertile and varicocele groups respectively and the difference was significant($p = 0.000$) and also there was a significant difference between three groups with regards to Acridine orange test results (16.7±8.55, 33.50±9.58, 60.85±15.61 in control, infertile and varicocele groups respectively and $p = 0.000$). Recent study demonstrated that the varicocele samples contain a higher proportion of sperm cells with abnormal DNA and immature chromatin than those from fertile men and also from infertile men without varicocele. In conclusion, we can say that varicocele cause the

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production of spermatozoa with less condensed chromatin, and this is one of the possible causes of infertility due to varicocele.

P29 (Poster presentation 29): Detection of *Chlamydia* trachomatis infection in infertile women by PCR using swap versus spatula

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Chlamydia trachomatis is one of the most common sexually transmitted pathogens of humans, with an estimated 92 million new cases occurring worldwide each year C. trachomatis often causes asymptomatic genital tract infections in both men and women, and the high number of unrecognized infected individuals provides a reservoir for spreading the infection to men and women via sexual contact. Aim of this part of study was to compare the sensivity of sampling using swap and spatula. Two specimens from 67 infertile women attending the Research & Clinical Centre for Infertility, Yazd-Iran, were examined by the PCR for the presence of Chlamydia trachomatis by specific paired primer (144 pb) sequenced as Sense: 5'CCT GTG GGG AAT CCT GCT GA A 3' and antisense 5'GTC GAA AAC AAA GTC ACC AT A GTA 3' for MOMP. Serum from each patient was also obtained and analyzed for the presence of IgG and IgA *Chlamydia*l antibodies by ELISA. *C. trachomatis* was detected by PCR in 0.7% of Spatula Ayre plus PBS samples and none of swap plus SP2 buffer samples was positive, $p < 0.01$. It should be recommended using spatula Ayre for detection of C.*trachomatis* in infertile couples.

P30 (Poster presentation 30): Religious beliefs role on reproductive heath behavior

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Human population growth poses a serious danger to the environment and consequently to human society itself. Land, water and air pollution, depletion of natural resources, soil erosion, poverty, sickness and distress. It often leads to malnutrition and high mortality rate in mothers and children. Where this exists, mothers with iron deficiency and children cannot learn, because of the lack of proper nutrition. Child growth is related to: Nutritional status of a population, socioeconomic, health, water or sanitation. Children are assets of any country. Reproductive health not only prevents births but also saves the lives of women and children. It is unthinkable that women should be left to shoulder this responsibility alone. Most pregnancy prevention programs focus on young women, although reproduction is a dual commitment but so often in much of the world, it is seen as wholly Women's responsibility. Concentration on men like women is obviously an inevitable necessity. The religious leaders play key roles in enhancing the attitudes towards and practice in reproductive health. Reproductive health should also use the curriculum to educate religious leaders who in turn will educate their societies. Sistan & Baluchistan province (Iran) where this research carried out is located in south –east of Iran. This area has been known with highest fertility rate and infant mortality rate in the

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country. The main aim of the study was to assess knowledge, attitude and practice of traditional religious leader's male teachers about reproductive health. A descriptive cross – sectional survey using a self-administered anonymous questionnaire which covered all aims our study were given to respondents to fill out voluntarily. The attitude mean appropriate ages for marriage: 16.3±2.43 for girls. On the whole 90.3%claimed the appropriate ages for girl marriage are less than 18 year and 6.8% said less than 12 year. 51.5 % claimed that they prefer have more children in the future.89%reported the appropriate age for pregnancy is under18 year and only 6.24%reported 20year.40%pregnancy interval less than two year. 3% subjects had over 10 children and 36.6% causes of their children deaths were infections.about50% reported that the men decide for pregnancies. Between knowledge and age variable were significant (p=0.029, r=-0.143).The study shows most pregnancy prevention programs focus on young women; few effective or innovative programs exist for adolescent males. Most of times, men decide for pregnancies. In many developing countries, every death (infant mortality) brings the next birth a little nearer. The preference for sons is deeply rooted in patriarchal tradition. Young mothers, particularly when not supported by the husband, may lack the economic means and practical knowledge to care well for their children. The global reproductive health targets without community participation especially religious leader's cooperation in some parts in many developing countries will not be achieved. In the indigenous populations many problems still exist: high birth and mortality rate, the preference for sons, pregnancy at low ages. If clients have a better understanding of fertility awareness, they are in a stronger position to make informed decisions about how they wish to manage their reproductive health. It is recommended that reproductive health education be discussed openly in traditional religious training centers and included in curricula.

P31 (Poster presentation 31): Evaluation of the impressive effect of Pentoxifylline on WBC count in serum and peritoneal fluid and also the growth of endometriotic implants in female rats.

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Endometriosis is defined as the growth of gland and stroma of endometrium outside of the uterus in ectopic places. Disturbances in immune system have an important role in this disease which may lead to infertility. It seems that inflammatory cytokines and specially TNF-α, which produced by activated macrophages have an important role in pathology of endometriosis. Based on this theory, anti TNF-α drugs are suggested as new drugs for Endometriosis. This experimental study has been done on female rats to determine the impressive effect of Pentoxifylline on white blood cells (WBC) count in serum and peritoneal fluid and also on the growth of endometrial implants. In proestrus phase, one horn of rat's bicorn uterus was removed surgically and the endometrium implanted to different places as follows: subcutaneous, peritoneum and near ovaries. After two months observation, female rats divided

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into two groups randomly. In treated group (n = 10) Pentoxifylline and in control group (n = 10), normal salin was injected subcutaneously. Then second laparotomy operation was performed and the size of implants and the amount of WBC in serum and peritoneum was measured. In treated group, the size of implants was decreased significantly in right subcutaneous (p = 0.01), left subcutaneous (p = 0.01), right ovary (p = 0.001) and left ovary (p = 0.005). In treated group, the total WBC count (p = 0.02) and neutrophils (p = 0.000) in serum were decreased and the total count of lymphocytes (p=0.003) in serum were increased. There weren't any significant changes in the total count of WBC in peritoneum in both groups. The number of Estrus cycles in both groups was similar. Based on our study, Pentoxifylline can decrease the size of endometrial implants especially in ovaries and subcutaneous areas and total WBC count in serum. Pentoxifylline can increase the lymphocyte count and decrease the neutrophil count in serum and by these changes it can alter the immune system. Pentoxifylline didn't have any adverse effect on rat's cycles and a good aspect of treatment with Pentoxifylline is achieved.

P32 (Poster presentation 32): Embryo transfer: ultrasound-guided versus clinical touch

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Our aim is to investigate the effect of ultrasound-guided embryo transfer on the rate of pregnancy. A prospective randomized trial is performed to compare ultrasound-guided embryo transfer with the traditional method. A total of 180 patients will be randomly divided into two groups on the day of embryo transfer. For the cases, ultrasound-guided will be used; controls will be performed using routine methods. From 180 patients, 72 of them (in case group: 39 and in control group: 33) have been gathered until now. Implantation and clinical pregnancy rates are similar in both groups (p > 0.05).

P33 (Poster presentation 33): The capacity of different human embryonic stem cell lines to generate trophoblast under various conditions *in vitro*

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Abnormal implantation of the human embryo in the uterine endometrium can lead to poor fetal development and miscarriage, or the potentially life-threatening condition, pre-eclampsia. The knowledge we have to date about trophoblast invasion is limited due to ethical and practical restrictions. Also, implantation in laboratory animals is markedly different to that in women. Here we investigate the capacity of human embryonic stem cell (hESC) lines derived in Sheffield to differentiate to trophoblast stem cells. Our long-term objective is to develop and validate an in vitro model with human trophoblast stem cells to investigate molecular events in early stages of human implantation. HESCs were treated with collagenase, aggregated into embryoid bodies (EBs) in serum replacement medium for 5 days and transferred into 96-well plates in trophoblast stem cell conditioned medium (TSCM) for 24 hours. Supernatant from each well was

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collected and tested for human chorionic gonatotropin β (hCGβ) secretion using sandwich enzyme-linked immunoassay (ELISA). EBs expressing high levels of hCGβ were selected, passaged in TSCM and checked regularly for the hCGβ secretion. In another protocol, clumps of HESCS were treated with Trypsin-EDTA solution to generate single cells that were transferred to medium with the growth factor bone morphogenetic protein 4 (BMP4, 10 ng/ml) on feeders and Matrigel. Cells were assessed for hCGβ, HLA-G and CK7 expression of trophoblast differentiation by immunostaining. In comparison to the Wisconsin H7S14 cell line (200-500 mIU), Sheffield lines S4, S1 and S6 exhibited lower hCGβ levels (50 mIU). EBs with low but significant hCGβ secretion was grouped together for passage. These cells exhibited multinucleated cells and displayed staining for CK7 and HLA-G. Cultures in the presence of BMP4 in Matrigel showed multinucleated cells on day 6 and hCGβ expression indicating trophoblast characteristics. On days 10 and 12, high hCGβ secretion was expressed in cells cultured with BMP4 and on feeders. The results suggest that there is a difference in the inductive capacity of hESC lines to differentiate into trophoblast cells.

P34 (Poster presentation 34):
The effect of necrotic blastomere removal on Vitrified – Warmed 4-cell stage mouse embryos development.

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The cryopreservation of embryos has become an integral part of ART. The aim of this study was evaluating the necrotic blastomere removal effect on vitrified – warmed 4-cell stage mouse embryos development. The retrieved 2-cell mouse embryos were cultured in G_1^{TM} ver3 medium until the 4-cell stage, then the embryos were vitrified with pretreatment and vitrification solutions. After warming the embryos were transferred to G_1^{TM} ver3 medium, and divided into intact (control) and partially (25%, 50% and 75%) damaged groups. In the partially damaged groups, the necrotic blastomeres were removed after zona pllucida laser hatching and embryos of two groups (control and removal) after reached to 8-cell stage were cultured for additional two days into G_2^{TM} ver3 medium. Finally, the rate of blastocyst formation, number of total blastomeres and number of blastomeres in each part of the inner cell mass (ICM) and trophoectoderm (TE) and apoptotic cells were compared statistically using χ^2 and ANOVA tests. The blastocyst formation was increased in all partially damaged groups after removal of necrotic blastomeres. This increase was only significantly ($p < o.o1$) in partially 75% damaged group. Also, the apoptotic cells were decreased significantly in all partially damaged groups after necrotic blastomere removal. The necrotic blastomere removal improved development and quality of partially damaged vitrified – warmed mouse embryos.

P35 (Poster presentation 35):
Effect of retinoic acid on maturation and development of immature mouse oocytes and resulted embryo from their fertilization in vitro

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The purpose of this study was to evaluate the effect of different doses of retinoic acid on resumption of meiosis, in vitro maturation of oocytes and resulting embryo development in mice. Germinal vesicle of NMRI female mouse (6-8 weeks old) oocytes were collected from ovaries and cultured in maturation medium MEM-α contained: 100 mlIU/ml rFSH + 7.5 IU/ml hCG + 5% FCS (Control group). All- trans retinoic acid (t-RA) at concentrations of 0.25, 0.5, 1, 2 μM and ethanol alone (Sham group) 0.2% (v/v), were added to oocytes maturation medium. After 24 hours the matured oocytes were inseminated with spermatozoa in T6 medium and cultured for 5 days. The retinoic acid supported progression and resumption of meiosis and also increased advancing the oocytes to Metaphase II, formation of morula and blastocyst compared to control group. The difference between our experimental groups and control in resumption of meiosis increased by t-RA dose from 0.25 μM ($P < 0.01$) to 0.5 μM ($P < 0.001$), 1 μM ($P < 0.001$) and 2 μM ($P < 0.0001$), but higher doses like 5 μM and 10 μM reduced it, so was not continued and was excluded from our doses. The rate of oocytes developing to the Metaphase II stage of maturation was significantly increased with t-RA doses of 1 μM ($P < 0.01$) and 2 μM ($P < 0.0001$) comparing to control group. The rate of embryos reaching to morula after 72 hours was significantly increased with 1 and 2 μM t-RAin ($P < 0.0001$). The rate of embryo that developed to blastocyst stage was significantly increased in medium containing 2μM retinoic acid in comparison to other groups ($P<0.01$).We found that All- trance retinoic acid enhanced mouse oocytes maturation in vitro and improved embryo development in a dose dependent manner.

P36 (Poster presentation 36): Effect of growth hormone Administration after methotrexate on rat testicular tissue

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Methotrexate (MTX) is a chemotherapeutic agent that used for the treatment of a variety of tumors and inflammatory, diseases. Methotrexate cause defective oogenesis and spermatogenesis, and impair fertility. the aim of this study was planed to see the role of human growth hormone (hGH) on testis recovery after treatment with methotrexate in rat .the fifty male wistar rat were selected and randomly divided into five groups; control (n=10) and test (n=40).GH group was received 0/3 mg/kg hGH (IP) for 28 consequence day ,MTX group was received 1mg/kg MTX (IP) per week for four consequence week ,GH/MTX group was received 0/3mg/kg hGH(IP) for28 consequence day plus 1mg/kg MTX(IP) per week for four consequence week and MTX/ post GH group was received 1mg/kg MTX (IP) per week for tow consequence week With 0/3mg/kg hGH (IP) for 14 consequence day ,however the control group just received vehicle (IP) .in 14 and 28 day five rats from each group were sacrificed and the testis tissue of rats in whole groups were removed and sperm was collected from epididymis then prepared for analysis. This study was confirmed methotrexate had destructive effects in testis germinal cells and rate of spermatogenesis were decreased ($P<0.05$) as compared with control group, and hGH had treatment

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effects in degenerative seminiferous epithelium and improves spermatogenesis (P‹0.05) as compared in other groups. There were no significant differences in testicular and body weight and (number, motility and viability) of spermatozoa between GH and control group. There were significant decrease in testicular and body weight and (number, motility and viability) of spermatozoa between GH/MTX and control group. There was significant decrease in testosterone concentration between GH/MTX and MTX/post GH groups. These results suggested that hGH had recovery effect on spermatogenesis cycle after treatment with MTX, in contrast with simultaneously administration. Possibly hGH inducement is suitable for sperm health ability parameters after chemotherapy with MTX in patients.

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