Abstracts of
Royan International Twin Congress:
8th Congress on Reproduction Biomedicine
3rd Congress on Stem Cell Biology and Technology

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Guide for Authors

Yakhteh Medical Journal (The Cell) is a publication of Cellular Sciences Research Centre, Royan Institute. It is published both in Persian and English. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on exclusively cellular, molecular and other related topics. Yakhteh Medical Journal (The Cell) has been certified as a quarterly publication by Ministry of Culture and Islamic Guidance in 1999 and was accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission in 2000.

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A- Original articles are scientific reports of the original research studies. The article consists of Abstract (English & Persian), Introduction, Materials and Methods, Results, Discussion, Conclusion, acknowledgements and References.

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IN THE NAME OF GOD

Gone But not Forgotten

In the memory of the late Director of Royan Institute, Founder of Stem Cells Research in Iran and Chairman Manager of Yakhteh Medical Journal. May he rest in peace.

Dr. Saeed Kazemi Ashtiani

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Abstracts of Royan International Twin Congress: 8th Congress on Reproduction Biomedicine 3rd Congress on Stem Cell Biology and Technology

Congress Chairman:
- **Hossein Baharvand, Ph. D.**

Congress Honorary Chairman:
- **Ariff Bongso Ph.D.**

Contents:

Scientific Board

Congress Chairman
Hossein Baharvand, Ph. D.
Associate Professor
Royan Institute, Tehran, Iran

We would like to extend a warm welcome to all the participants at 8th congress on Reproductive Biomedicine and 3rd congress on Stem Cells Biology and Technology in Tehran. We expect the congress to provide all of you with the best of science and education. This is a unique forum where scientists can meet their colleagues and exchange ideas and information in the field of reproductive biomedicine and stem cells biology and technology.

The scientific programme has been prepared by the Local Scientific Committee, incorporating suggestions of the International Advisory Committee regarding the selection of topics and speakers. Both these committees working in collaboration have been beneficial for everybody, and have contributed to the successful preparation of the programme. We have endeavored to include most of the different aspects that make up reproductive biomedicine and stem cells biology and technology in the programme. Given the complexity of these fields and given the numerous links with biology and other fields of medicine and molecular and cellular biology, it is not easy to include all subjects. Nevertheless, the offer is wide and I am sure that each of you will find something of interest.

Abstract submissions again exceeded all expectations and have been accommodated by further increasing the number of posters. Several highly specific and cutting-edge researches will be presented by scientists from national and international institutes and universities.

Social interaction has always been a most enjoyable part of 8th congress on Reproductive Biomedicine and 3rd congress on Stem Cells Biology and Technology congress, where physicians, nurses, geneticist, cell and molecular biologists and all kinds of health professionals dealing with reproductive biomedicine and stem cells come together not only to present and discuss topics of interest but to enjoy the relaxed atmosphere and social programme offered.

I hope that this congress will be a memorable experience for all the participants and at least as fascinating as it has been for those responsible for the scientific programme.

Hossein Baharvand

Congress Honorary Chairman
Ariff Bongso Ph.D.
Research Professor,
Yong Loo Lin School of Medicine,
National University of Singapore,
Kent Ridge, Singapore

Firstly, I would like to thank the Royan Institute for inviting me as Honorary Chairman of this congress. On behalf of Professor Hossein Baharvand, the organizing committee and the Royan Institute I would like to welcome all overseas and local participants to the Royan International Twin Congress on Reproductive Biomedicine and Stem Cell Technology. The Royan Institute has been very active in both these disciplines and has been successfully organizing such meetings regularly. The twin theme for this congress is quite appropriate as the discipline of reproductive biomedicine evolved naturally into the discipline of embryonic stem cell biology. Embryonic stem cell biology has benefited from the rapid advances in clinical embryology and it is interesting that now clinical embryologists are learning from the findings of embryonic stem cell biology in their quest to enhance birth rates in childless couples. The field of stem cell biology in itself has become very wide with excellent work in progress on a variety of stem cell types ranging from the preimplantation embryo to germ cells to the fetus and the adult. This meeting will attempt to bridge the gap between stem cell biology and reproductive biomedicine by covering novel aspects of work in these areas by very eminent scientists whose noble quest is to seek cures for the many diseases that plague mankind.

I am overwhelmed at the wide spectrum of overseas and local speakers who have agreed to participate and share their experiences and knowledge at this forum. This is a great opportunity for Iranian doctors and scientists to interact and benefit from the experiences of their overseas counterparts who are giants in their respective fields. I am hoping there will be a lot of interaction and sharing of information. I am sure our overseas guests will also enjoy the overwhelming hospitality of the organizers, the Iranian people, and this beautiful country.

I would like to extend my personal thanks on behalf of all the overseas participants to Professor Hossein Baharvand and his organizing committee for inviting all of us. I wish all of you a very successful congress.

Ariff Bongso

Oral Presentations

Andrology

O-1: The Role of Color Doppler Ultrasound in Prediction of the Outcome of Microsurgical Subinguinal Varicocelectomy

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Objective: Over-diagnosis and under treatment of varicocele may be responsible for poor outcome of varicocelectomy. In this study, we used Color Doppler ultrasound for accurate diagnosis and grading of varicocele and to predict the outcome of microsurgical subinguinal varicocelectomy.
Materials and Methods: A total of 104 patients undergoing microsurgical subinguinal varicocelectomy for treatment of infertility were included in this study. Patients were evaluated with routine history, physical examination, semen analysis, hormonal assessment and scrotal ultrasound and Doppler. After varicocelectomy, improvement index, in sperm concentration, was calculated by dividing the difference between the postoperative and preoperative sperm concentration by the preoperative sperm concentration. Improvement Index greater than 0.5 is considered a good outcome. Statistical analysis was done to study the correlation between microsurgical varicocelectomy outcome and testicular vein diameter at the inferior pole of the testis and the degree of reflux measured by color Doppler ultrasound.
Results: Improvement index in sperm concentration, motility and morphology more than 0.5 was achieved in 58.8%, 27.3% and 17.6% of cases respectively. We found that patients with testicular vein diameter, at the inferior pole of the testis, more than 2.5 have significantly higher improvement index in sperm concentration, motility and morphology than in patients with testicular vein diameter less than 2.5mm (p=0.006, 0.016, 0.041 respectively). We also found that patients with clear reflux detected by color Doppler ultrasound at the inferior pole of the testis have a significantly higher improvement index in sperm concentration, motility and morphology than patients with reflux detected only in the supratesticular venous channels (p=0.013, 0.015 and 0.045 respectively).
Conclusion: Color Doppler ultrasound is a useful tool for accurate diagnosis and grading of varicocele and to predict the outcome of varicocelectomy. We recommend doing varicocelectomy in cases of testicular vein diameter more than 2.5mm and in cases of reflux detected at veins at the lower pole of the testis.

O-2: Human Sperm and DNA Damage: Modern Trends of Andrology

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Functions of seminal vesicle, prostate and bilateral testes can generally be determined by parameters of sperm analysis such like concentration, motility, morphology, semen volume, and pH. Infertile men frequently have lower sperm parameters than their fertile counterparts. However the results of sperm analyses among these groups are sometimes overlapping. As well traditional spermiogram and classical morphological assessment even based on strict morphological criteria sometimes fail in certain prediction of reproductive outcome of infertile men who undergone artificial reproductive techniques with their partners. Thus new and more sensitive methods, especially for detecting sperm function and viability, were being needed by many experts. Human sperm DNA is one of the most attractive targets for majority of experts during advances. Therefore various clinicians assumed that sperm DNA might comprise many useful clinical parameters and predictors for reproductive outcome. Conversely to loose structure of chromatin (DNA and nuclear proteins) in somatic cells, chromatin is tightly compacted due to unique associations between the DNA and sperm nuclear proteins.

It has been shown that in cases of DNA damage nuclear protein ratios determined as protamine and histone ratio altered favoring histone resulting in loose package of sperm DNA. Different from nuclear DNA small and circular mitochondrial DNA is also structured in sperm mainly reflecting maternal inheritance and linked with sperm motility. Sperm DNA causes, much like cause of male infertility, have many factors such as reactive oxygen species and their damage, febrile disease, testicular hyperthermia, varicoceles, drugs, chemotherapy, radiation, genital tract infections, environmental causes, genetic deficiencies and hormonal factors. Nevertheless DNA damage of sperm mainly related with male infertility. There are several tests such as TUNEL, COMET assays, sperm chromatin structure assay and nuclear protein composition with protein separation. All of these tests verify sperm DNA damage and its ratio or its link with male infertility and sperm function. Majority of their mechanism are showing DNA breaks and others examine either ratio of protamine / histone or the susceptibility of sperm DNA to denaturation. Increased sperm DNA damage has been shown precisely to be related with poor reproductive outcome in natural cycles. As well it has also been reported that naturally sperm with damaged DNA are unlikely to attached fallopian tube epithelium resulting in natural selection. However IVF and ICSI bypass this selection leading oocyte fertilization by sperm with mild and moderate DNA damage. Therefore some of the studies those included IVF and ICSI patients indicated only fertilisation failure whereas some of them reported poor embryo quality and lower ongoing pregnancy rates. More or less the studies met some topics that till four cell stage embryonic genome is not activated and resulting in unaffected embryonic morphology beyond this stage. As well today there are some attitudes on recovery of sperm DNA damage by oocyte after fertilisation forming healthy embryonic genome up to several damage degrees. However majority of these studies indicated that embryonic quality beyond 4 cell stage, ongoing pregnancy and fertilisation rates less or more linked with sperm DNA damage. Therefore sperm DNA damage is closely related with reproduction either naturally or with ART. Conclusively sperm DNA damage without any doubt should be included in the current and advanced management of infertile couples. In future sperm DNA damage seemed to have a severe role in assisted reproduction especially in selection of treatment choice.

O-3: Semen Analysis-WHO Manual 5th Edition and the Future of Automation

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WHO has made a significant contribution by producing successive revisions of the semen analysis manual. The 5th edition to be published soon has a number of improvements with clearer and simpler instructions for standard analysis particularly for sperm concentration and motility. Morphology continues to be done by the strict method. There are tables for comparing duplicates. New reference values (weighted 5th percentiles) from about 1600 fertile men (recent fathers) from different countries are included. The section on sperm preparation has been expanded to cover common methods of isolation of sperm for assisted reproductive technology. A chapter on sperm cryopreservation has been added. The quality control chapter has been rewritten. There is less material in appendices but there is a new appendix on the basics of microscopy. The aim of the manual is to standardise semen analysis but experience with external quality assurance indicates this is difficult to achieve. This is particularly the case with sperm morphology. Clinicians will continue need to know the reference ranges for their own laboratories. We have experience with routine use of computer assisted semen analysis (CASA) for sperm concentration, percent progressively motile sperm and sperm kinematics: straight line velocity (VSL), using the Hamilton Thorn CASA with IDENT fluorescent staining of the sperm. This requires dilution of samples with high sperm

concentration with seminal plasma and selection of fields across the counting chamber to achieve accuracy. For sperm concentrations above $2x10^6$/mL the quality control is very good. We have a sperm morphometry system based on automated focusing and slide movement that provides 32 morphometric measurements of the sperm head and upper neck regions including features related to density of staining that allow orientation of the sperm head and assessment of the acrosomal region. An index (%Z) derived from features selected by the sperm-ZP binding process was related to natural conception rates in about 1200 subfertile couples. VSL and female age were also significant in regression analysis models. We believe these and other advances in CASA will greatly improve semen analysis.

O-4: Human Sperm-Oocyte Interaction Testing

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During natural conception and standard in vitro fertilisation (IVF), motile capacitated sperm with intact acrosomes bind to the surface of the zona pellucida (ZP) and this binding triggers the acrosome reaction (AR). The sperm then passes through the ZP and binds to the oolemma via the plasma membrane that persists over the equatorial segment. The sperm is then engulfed into the ooplasm where decondensation of the nucleus occurs to form the male pronucleus. We have developed tests for human sperm-ZP binding, the ZP-induced AR and sperm-oolemma binding using oocytes which failed to fertilise in the clinical IVF program. The patients consent to the use of this material for testing or research. Usually the oocytes maintain their ability to bind sperm and stimulate the AR. The ZP can be preserved in concentrated salt solution at 4^oC for months. Using these assays we have found defective sperm-ZP binding and disordered ZP-induced AR are common causes of failure of IVF when there are sperm defects, but can also occur with normal semen analysis. These defects of sperm-oocyte interaction could account for about 25% of patients with idiopathic infertility and if diagnosed before IVF is attempted would allow the patients to be treated by ICSI and avoid an IVF cycle with low or zero fertilisation. In contrast, oolemma binding defects appear to be rare. Using experimental conditions in which the amount of ZP was not limiting, we showed that only a small proportion (<25%) of motile sperm in the semen of fertile men is capable of binding to the ZP and this was correlated with sperm morphology. The strong relationship between morphology and sperm ZP binding led us to study the morphological characteristics of ZP preferred sperm and quantified this by computer image analysis of sperm morphometry. We have also investigated the role of protein kinase C, actin and other signal transduction and membrane fusion molecules in the ZP-induced AR. We have been unsuccessful in producing active recombinant human ZP proteins. Currently we are trying to identify possible sperm receptors for the ZP. In the future, simpler alternative tests of sperm functional capacity that correlate with sperm-oocyte interaction will be developed that should assist in the prognosis for natural conception of couples with mild abnormalities of semen analysis such as isolated teratozoospermia and idiopathic infertility. Such tests should also help assign patients to IVF or ICSI.

O-5: Modern Management of Male Infertility

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Males suspected to be infertile should have a detailed medical history and physical examination. Semen analysis is the main investigation. Unless the clinical picture is clear, several semen analyses need to be done because of the day-to-day variability. Measurement of gonadotrophin and

testosterone levels is helpful in distinguishing primary from secondary testicular failure. Testis biopsies are useful for confirming obstructive azoospermia and determining the type of spermatogenic defect with primary seminiferous tubule disorders. Other investigations: karyotype, genetic tests for Yq microdeletions, cystic fibrosis, imaging for pituitary tumour or ejaculatory duct obstruction, are performed where indicated. A number of conditions are untreatable and cause sterility, in particular primary spermatogenic disorders where no live sperm are produced. These patients need to consider other alternatives for having a family by donor insemination or adoption. Over the last 15 years it has become clear that sperm or elongated spermatids that can be used for intracytoplasmic sperm injection (ICSI) may be found in a proportion of patients with severe testicular disorders such as Klinefelter syndrome and Sertoli cell only syndrome, either in the semen or in testis biopsy specimens. Conditions that may be treatable to increase the chances of natural conception include: gonadotropin deficiency or suppression, sperm autoimmunity, genital tract obstruction and reversible toxin exposures or illness effects and some coital disorders. However ICSI is often a better alternative for conditions such as sperm autoimmunity and genital tract obstruction. The remaining patients have less severe abnormalities of the semen, ranging from oligospermia to normal standard semen analyses but defective sperm function. These patients may have varicoceles, features of low grade genital tract inflammation, increased abnormal DNA in the sperm and increased production of reactive oxygen species by their sperm. There are no good controlled trials which prove treatment of these problems will increase natural conception rates. These patients are subfertile rather than sterile and pregnancies may occur but at lower than normal rates. Infrequent or mistimed coitus and female factors such as ovulatory disorders may be contributing. Thus the male and female partners should be treated as a couple and reversible factors treated where possible. The estimation of prognosis for natural conception is also important. If this is low, ICSI is usually effective.

O-6: Signal Transduction Mechanisms Involved in *In Vitro* Ram Sperm Capacitation

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This article represents a step forward in our study on ram sperm functioning and the relationship with fertility. Artificial insemination in sheep has not been widely adopted, probably due to the low fertility rate obtained with frozen-thawed semen, possibly due to premature capacitation-like changes. Therefore, a good knowledge of the sperm capacitation process could help in the formulation of better diluents that prevent these changes during sperm freezing or storage, and, therefore, improving the sperm fertilizing capacity.
In previous studies we reported that *in vitro* capacitation and acrosome reaction induced a decrease in the content and the redistribution of P14 and P20, two ram seminal plasma proteins that protect spermatozoa against cold-shock. Our results suggested that the protective effect of these proteins could be related to their decapacitating role (JAndrol2005). Likewise, we showed that membrane protein tyrosine phosphorylation is related to the capacitation state of ram spermatozoa (MRD2001). However, there had not been any report about the molecular regulation mechanism of this process in ram. Therefore, in this study we investigated basic aspects of the signal transduction pathways that are activated during capacitation. Our results demonstrated that in ram sperm, capacitation and the associated protein tyrosine phosphorylation is not absolutely dependent on the presence of BSA and calcium, and that the PKA/cAMP pathway is, at least, partially implicated in the tyrosine phosphorylation of some proteins.
Our data indicate that the signal transduction mechanisms of capacitation in ram sperm differ from those in other mammals, which suggests that species specificities might exist

with respect to this process. Our findings might benefit our understanding of the biochemical mechanisms involved in mammalian sperm capacitation and ultimately, fertility.
Furthermore, in this article we validate the chlortetracycline (CTC) technique for the assessment of ram sperm capacitation state, performing a specific determination in viable cells exclusively.

O-7: Hyaluronan Binding Protein (HABP1) as Diagnostic Marker for Male Infertility and its Use in IVF

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The lack of effective diagnosis of testicular disorders leading to infertility is a major problem in reproductive biomedicine. A better understanding of male infertility related to abnormal spermatogenesis associated with differential gene expression is of interest. We are working on a novel hyaluronan binding protein (HABP1) that interacts specifically with HA and facilitates HA mediated processes including sperm-oocyte interaction1 and sperm motility2. Sequence analysis of HABP1 from human fibroblasts reveal its identity with SF2/p32, the protein copurified with alternate splicing factor3 and globular head of C1Q thus represented in human Chromosome 17p13.34 as synonym HABP1/p32/C1QBP. HABP1 is synthesized as a proprotein which forms mature protein by cleavage of initial 73 amino acids. This proprotein form is extremely labile and detected only in pachytene spermatocytes and round spermatids in germ cells in adult testis5. Further analysis demonstrates that though mature form of HABP1 is present in the testis, its precursor form was not found in testis of 7, 14, 21 and 28 day old rat, but is present only in pachytene spermatocytes and round spermatids of testis of 21 day and 28 day old rats. With spermatogenic arrest, HABP1 is lost from pachytene and round spermatids suggesting that the expression of HABP1 proprotein may be crucial for spermatogenesis6. Earlier we reported on the reduction in the level of HABP1 on sperm surface in asthenozoospermic and oligospermic patient7. In continuation, we have also demonstrated the interaction of HABP1 with zona pellucida of buffalo and shown that the supplementation of IVF medium with rHABP1 can promote the capacity of sperm binding to oocytes under invitro fertilization conditions even in presence of D-mannosylated albumin (DMA), known to inhibit sperm oocyte interaction8. Thus we propose to use HABP1: 1. as a diagnostic marker for male infertility and spermatogenic arrest in testicular biopsy.
2. in IVF medium to promote sperm oocyte interaction.

O-8: Correlation between Seminal Plasma Glutathione Peroxidase Enzyme Activity and Semen Parameters

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Introduction: Sperm cell membranes are susceptible to peroxidative damage by an excess of reactive oxygen species (ROS). Antioxidative defence systems consisting of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) physiologically control the balance between ROS production and neutralization. **Materials and Methods:** In the present study, correlations between seminal plasma glutathione peroxidase enzyme activity and

semen parameters are evaluated in 240 males. Semen analysis was performed according to World Health Organization guidelines. The 240 males were subdivided into 5 groups as normospermia, oligozospermia, asthenozospermia, azospermia and varicocele according to their spermiogrames. Seminal plasma glutathione peroxidase enzyme activity was determined by Kit (Randox, Germany).
Results: The result showed that glutathione peroxidase enzyme activity is higher in normospermic than oligozospermia, asthenozospermia, azospermia and varicocele groups. Also, there are significant and negative correlations between glutathione peroxidase enzyme activity and seminal plasma fructose concentration, white blood cell, tail defects of sperm, coiled tail sperms and short tail sperms. On the other hand, the present data showed that significant and positive correlations between vitality, sperm count, motility and normal morphology.
Conclusion: So, the present study showed that measurement of glutathione peroxidase enzyme activity could be a good marker for evaluation of male infertility.

O-9: Comparison of the Number of Spermatogonia and Sertoli Cells in Fetal and Neonatal Testes Autopsied between 1958-1964 and 1989–1998 in Tokyo

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Background: Studies in several countries have reported a decline in human sperm quality similar to that observed in wild animals. To quantify whether the number of sperm in humans has decreased and whether humans are affected by similar environmental influences, we compared the number of spermatogonia and Sertoli cells in human fetal and neonatal testes autopsied at two institutions in Tokyo between 1958–1964 (term A) and 1989–1998 (term B), with special attention to chronological changes during gestation.
Materials and Methods: We used an immunohistochemical method with antibody against neuron-specific enolase to determine the percentage of seminiferous tubules containing spermatogonia in the formalin-fixed tissue samples, and a morphometrical method using a dissector to count the number of spermatogonia.
Results: There were no significant statistical differences between the two time periods in the regression parameters compared for the number of spermatogonia and Sertoli cells, nor was there a remarkable difference in the estimated number of Leydig cells.
Conclusion: The results indicate that even if there has been a deterioration in human semen quality, it is not necessarily caused by endocrine disruption of fetal testicular development.

O-10: The Effect of Semen Parameters and Sperm Chromatin Status before and Post Varicoclelctomy on Fertility

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Introduction: Different methods have been used to evaluate the beneficial effect of varicocelectomy; these include semen parameters and pregnancy rate. Due to high biological variability of semen parameters, sperm functional tests have been considered as an efficient end point in assessment of fertility. Therefore, the aim of this study was to evaluate the effect of varicocelectomy on semen parameters, sperm chromatin and membrane integrity.
Materials and Methods: In 35 patients over a 2 year period protamine deficiency, presence of excessive histone, chromatin stability,

ability of sperm to undergo decondensation and membrane integrity were assessed by chromomycin A3, aniline blue staining , SDS, SDS+EDTA and HOST, respectively.
Results: The results of this study showed that among semen parameters only sperm motility, sperm membrane integrity and sperm chromatin integrity showed significant improvement post surgery. The cumulative pregnancy rate in this was 25.7%. Comparing the results of the aforementioned parameters between patients whom became pregnant compared to those who did hot benefit from varicocelectomy show that patient may benefit from varicocelectomy that have improved sperm morphology, decreased sperm protamine deficiency and decreased presence of excessive histone at 3 month post surgery ($p<0.05$).
Conclusion: These results suggest that the initial values and 3 month post varicocelectomy values of some of these functional tests may help specialist to foreseen the outcome of surgery and may help in patient management.

O-11: Comparison between Reactive Oxygen Spieces (ROS) Concentration in Seminal Plasma and Semen Parameters in Partners of Patients who Became Pregnant after IVF/ICSI and Those Who Did Not

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The aim of this study was to determine and compare the level of ROS in seminal plasma and other sperm parameters (count, morphology, membrane integrity, maturity, DNA-fragmentation) in order to find out the relationship between ROS concentration and spermatozoa quality and their effect on fertilization and pregnancy of patients who became pregnant and those who did not after IVF/ICSI treatment. 26 IVF and 22 ICSI patients were included in this study, the ROS level in seminal plasma and sperm concentration, vitality (Eosin-test), motility, morphology, membrane integrity (HOS-test), maturity (Chromomycine CMA_3) and DNA Fragmentation (TUNEL) results and their relationship to fertilization and pregnancy were analysed. ROS concentrations were at the same level in the seminal plasma of male partner of patients who became pregnant and those who did not. The other semen parameters, concentration, motility, vitality, membrane and DNA integrity were similar in both groups. However, in both groups a negative correlation could be found between ROS concentration and sperm vitality, membrane integrity and morphology. Besides an inverse correlation, could be found between TUNEL, vitality, and membrane integrity.
In Conclusions: ROS concentration in seminal plasma affects spermatozoa's quality but can not affect the fertilization rate in IVF/ICSI programme.

O-12: The Limitations of the Semen Analysis and the Importance of Diagnosis in the Evaluation of Infertility in the Male Patient

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Semen analysis is used both as an indicator of infertility as well as a basis for treatment. However a semen analysis gives poor discrimination between infertility and fertility and is only of real value in indicating infertility when the variables within the semen analysis are at their extremes.
The reason that a semen analysis is a poor discriminator of fertility is clear as the difference between a conception and a non conception cycle involves one single sperm: in a conception cycle all sperm are lost while in

a conception cycle all but one sperm are lost. Therefore the number of motile and morphologically normal sperm can only give the clinician a probability of pregnancy. Thus wether the count is 10% more or 10% less can make little difference clinically. For this reason accurate sperm counts are unnecessary in clinical andrology. Only when all the sperm in an ejaculate show an abnormality can a semen sample truly be said to be infertile.

One important aspect of infertility management is to arrive at a clinical diagnosis. Only with a clinical diagnosis can both the pathophysiology underlying patients infertility and the generation of an abnormal semen analysis be determined and understood. Only with an understanding of the causation of infertility can any preventative measures be taken, can any rational treatment be provided and the frequently unwarranted use of IVF be avoided. In the male, treatment often relates solely to the sperm count and not its underlying cause.

Much of the causation of male infertility in clinics today goes undiagnosed. In my recently completed (but unpublished) study of testicular ultrasound among a group of 749 men attending an infertility clinic, a large of amount, much of male infertility will remain misunderstood.

O-13: Raised Inflammatory Markers in Semen from Men with Asymptomatic Chlamydial Infection

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Introduction: 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.

Materials and Methods: 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.

Results: 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.

Conclusions: These results suggest that raised seminal IL-8 might be useful as a marker for silent male genital chlamydial

infection which in turn is associated with decreased sperm motility.

O-14: Comparative Effect of Aminoglycosides (Gentamycin, Neomycin, Streptomycin) and Fluoroquinolones (Ofloxacin) Antibiotics on Sperm Parameters and Testis Apoptosis in Rats

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Introduction: Aminoglycosides (Gentamycin, Neomycin, Streptomycin) and fluoroquinolones (ofloxacin) are synthetic antibacterial agent antibiotics with a very broad spectrum against microbial pathogens, especially the Gram-negative. The aim of this study was to investigate the comparative effects of these drugs on testis apoptosis and sperm parameters in rats.
Materials and Methods: The fifty male Wistar rats were selected and randomly divided into control (n=10) and experimental (n=40) groups. The experimental groups split into four groups. First, second, third and fourth experimental groups received 5 mg/kg (IP) Gentamycin, 50mg/kg (IP) Neomycin, 40mg/kg (IP) Streptomycin and 72mg/kg (PO) ofloxacin daily for fourteenth day, respectively; however, the control group just received vehicle (IP). In the fourteenth day, rats were killed and sperm removed from cauda epididymis and analyzed for sperm motility, morphology, and viability. Testis tissues were also removed and prepared for TUNNEL assay for detection of apoptosis.
Results: There was a significant decrease in sperm count, viability and motility in all of experimental groups when compared with control group ($p<0.05$). Although in streptomycin group these parameters were less decreased than in the other experimental groups.
The apoptotic cells were (24.15 ±10.17) in Gentamycin group, (25.15±9.11) in Neomycin group, (15.15 ±11.14) in Streptomycin group and (34.15 ±8.17) in ofloxacin group. These cells were significantly increased in the all experimental groups when compared with those seen in the control group (7.3 ±2.41) by ANOVA method ($p<0.05$).
Conclusion: Aminoglycosides (Gentamicin, Neomycin and Streptomycin) and Fluoroquinolones (Ofloxacin) antibiotics have negative effect on sperm parameters and testis apoptosis in rats. However, these side effects are less seen in the streptomycin group. Therefore, it is recommended that usage of this drug has fewer side effects on male fertility.

O-15: Effect of L-Ascorbic Acid Supplementation on Testicular Oxidative Stress and Endocrine Disorders in Mature Male Rats Exposed to Intensive Swimming Exercise

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In order to investigate the ameliorative potential of L-ascorbic acid on intensive swimming exercise induced testicular oxidative stress, 18 Wistar male rats (age: 3 months, weighing: 127.5±5.3g) were randomly divided into: 1) control group (CG, n=6); 2) experimental group (EG, n=6); 3) supplemented group (SG, n=6). An exercise protocol of 3 hour swimming/day, 5 days/ week was followed for 6 weeks in EG and SG with no exercise in CG. In SG L-ascorbic acid was supplied orally at a dose of 25-mg/kg-body weight/day for 6 weeks. A significant diminution ($p<0.05$) was noted, in paired testicular weights; epididymal sperm count; testicular Δ^5, 3β-hydroxyseroid

dehydrogenase (Δ^5, 3 β -HSD), 17β-hydroxyseroid dehydrogenase (17β-HSD); plasma levels of testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL); the numbers of preleptotine spermatocytes (pLSc), mid pachytene spermatocytes (mPSc) and stage 7 spermatids (7Sd) of stage VII seminiferous epithelium cycle in EG when compared to CG. A significant elevation ($p<0.05$) in plasma corticosterone and testicular content of malondialdehyde (MDA) along with significant reduction ($p<0.05$) in reduced glutathione (GSH), ascorbic acid, α- tocopherol, superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx), and glutathione-s-transferase (GST) were noted in testes of EG compared to CG. No significant change was noted in final body weight, numbers of spermatogonia-A (Asg) among the groups. Moreover, L- ascorbic acid supplementation restored the above parameters to the control level.

Conclusion: It may be concluded that intensive swimming exercise induced oxidative stress causes dysfunctions in male reproductive system, which can be protected by L- ascorbic acid.

O-16: Demonstration of Follicle-Stimulating Hormone Receptor in Cauda Epididymis of Rat

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FSH receptor has been shown to be specifically expressed only in the Sertoli cells in males. In one of our studies that consisted of deprival of endogenous FSH in immature rats and adult bonnet monkeys, atrophy of the epididymis was observed, cauda region being the most affected. Although epididymis is an androgen-dependent tissue, the changes in histology of the cauda region were observed without any associated change in the levels of testosterone in FSH-deprived animals. Considering this, it was of interest to evaluate the possibility of epididymis being a direct target for FSH action. In the present study, we have examined the expression of FSH receptor in the epididymis of rat and monkey. In the cauda region of rat epididymis, FSH receptor expression was demonstrated by RT-PCR and Northern and Western blot analyses. FSH receptor was found to be functional as observed by its ability to bind 125IoFSH, by an increase in cAMP production, and by BrdU incorporation following addition of FSH under in vitro conditions. These results suggest the possibility of a role for FSH in regulating the growth of the epididymis.

Episismis, follicle-stimulation hormone, follicle-stimulating hormone receptor, male reproductive tract.

O-17: Origin, Localization and Binding Abilities of Boar DQH Sperm Surface Protein Tested by Specific Monoclonal Antibodies

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Introduction: Seminal plasma proteins bind the sperm surface at ejaculation and may modulate several aspects of sperm activity during reproduction. DQH sperm surface protein, present in boar seminal plasma, shows affinity to phoshorylcholine, acidic polysaccharides, oviductal epithelium and zona pellucida glycoproteins.

Materials and Methods: Monoclonal antibodies (MAbs) against DQH protein were prepared and used for determination of the DQH protein origin in boar reproductive organs, its localization on boar spermatozoa, and for investigation of its binding abilities in the porcine oviduct and to the zona pellucida of the oocyte.

Results: The mRNA transcript of DQH protein was found in seminal vesicles, not in the testis, epididymis and prostate. Its translated products were immunodetected by MAbs in

seminal vesicle extract and fluid, on seminal vesicle tissue sections and on the membrane-associated acrosome part of ejaculated spermatozoa.

Conclusion: These results confirmed the ability of DQH protein to bind the sperm surface at ejaculation and to participate in the formation of the sperm reservoir in the porcine oviduct. Moreover, monoclonal antibodies reduced binding of sperm to oocytes and proved the role of DQH protein in the sperm-zona pellucida primary binding.

O-18: Expression of Stress Inducible Protein 1 (Stip1) in the Mouse Testis

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Phthalate esters are considered endocrine disruptors that interfere with the endocrine balance and development of the mammalian testis. Mono-2-ethylhexyl phthalate (MEHP), the active metabolite of the ubiquitously used plasticizer di-2-ethylhexyl phthalate (DEHP), acts upon Sertoli cells as initial target. By subtractive cDNA libraries we identified genes deregulated as response to MEHP in primary cultures of mouse Sertoli cells. The expression of mouse stress inducible protein 1 (Stip1) was detected as upregulated as a result of MEHP exposure. Stip1 is a cochaperone protein that is homologous to the human heat shock cognate protein 70 (hsc70)/heat shock protein 90 (hsp90)-organizing protein (Hop). To assess the presence and localization of Stip1 in mouse testis and its potential role in stress defense, we studied the expression pattern of the Stip1 protein by immunohistochemistry and of the mRNA by in situ hybridization. Both the protein and the mRNA of Stip1 were mainly found in the cytoplasm of all types of spermatogonia and spermatocytes up till zygotene, the expression decreased during late pachytene and was very weak in diplotene spermatocytes and round spermatids. Interestingly, this expression pattern resembled the pattern of stress sensitivity of spermatogenic cells in that the most sensitive cell types show the weakest expression of Stip1. This suggests an important role for Stip1 in the ability of germ cells to survive in stress conditions including high temperatures.

O-19: Reactive Oxygen Species (ROS) Level in Seminal Plasma of Infertile Men and Healthy Donors

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Introduction: Reactive oxygen Species (ROS) are a group of free radicals that in excessive amounts showed to have negative influence on sperm quality and function. Some Clinical entities such as varicocele, spinal cord injury and genital infections showed to be associated with high ROS levels in semen.In this study we assessed ROS levels in seminal plasma of infertile men and compare with its level in healthy donors. We also determined the ROS level in semen of infertile patients according to the etiology of infertility, and also the effect of smoking on its level.

Materials and Methods: We selected 95 infertile patients and 63 healthy donors as control. Complete physical examination, semen analysis, scrotal sonography and hormone assay were done for all patients. Azoospermic patients were excluded from the study. ROS level in semen was measured by chemiluminescence assay in both groups. Patients also divided in two groups according to the etiology of infertility.

Results: We were excluded 32 infertile patients from the study. Semen specimens from 126 individuals, 63 samples from infertile men and 63 from healthy controls were studied. The mean age of normal subjects and infertile men were 30.78±3.73 years and 31.43±6.60 years respectively. The mean

ROS level in normal men was 180.05 RLU, and 1852.04 RLU in infertile patients, which is significantly higher in case group (p=0.000). Fifty patients had varicocele and for the other 13 patients no any specific etiology was found. The mean ROS level in varicocele patients was 2215.42 and in unknown group was 454.44 (p=0.048).
Conclusion: Our 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.

O-20: Ultrastructural Study on Apoptotic Effects of Myleran in Spermatogenic Cells in Adult Mice Testis

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Introduction: Myleran is known to have testicular toxicity leading to male infertility. Little is known about ultrastructural effects of myleran on male germ cells. In this study, we examined the possible involvement of apoptosis in the induction of germ cells degeneration following treatment with different doses of myleran using transmission electron microscopy (TEM) and light microscopy (LM).
Materials and Methods: At the present study 8 weeks old NMRI mice were divided in 5 groups. Control animals were treated with DMSO. However the other animals in the 2nd, 3rd, 4th and 5th groups were treated intraperitoneally with different doses of myleran: 5, 10, 20 and 40 mg/kg respectively. All animals were dissected after35 days and their testis was evaluated for detection of apoptosis using TEM and LM.
Results: In all myleran treated groups except in 2nd group, the nucleous of spermatogonia was marginal hyperchromatin with a crescent form. Primary spermatocytes showed cellular shrinkage and vacuolization inside the cytoplasm. Presence of large spaces between adjacent cells was another sign. Few round spermatids were affected and all signs were increased with the increasing of the dose. In LM study spermatogonia numbers were significantly reduced in the 4th and 5th groups (p<0.001). Primary spermatocytes, round and elongated spermatids in the 3th, 4th and 5th groups were significantly reduced (p<0.001) too.
Conclusion: This study showed that myleran could induce apoptotic alterations in all germ cells mostly in spermatogonia and primary spermatocytes in the dose response. It is suggested that Myleran- Induced germ cells apoptosis may result in decreased spermatogenesis.

O-21: Effects of Different Doses of Hyaluronan on Human Sperm Motility, Vitality and Morphology

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Background: Important aspect of sperm function such as motility and capacitation appear to be mediated at least partially though hyaloronic acid (HA). The present study investigated effects of different doses of hyaluronan on sperm motility, vitality and morphology in human.
Materials and Methods: Motility, vitality and morphology of raw semen was analyzed according to WHO criteria before and 4 hour after treatment with different doses of hyaluronan (0, 750, 1000 and 1250µg/ml).
Results: The results of present study showed in the group treated with 1000 µg/ml hyaluronan compare to control group there was an increase in the percentage of stages 3 and 4 but a decrease in the number of stages 1 and 2. In the group treated with 1250 µg/ml stages 1 and 2 increased while stages 3 and 4 decreased. Vitality in all groups decreased except of the group treated with 1000 µg/ml hyaloronan. The group decreased. With 1250 µg/ml hyaloronan showed significantl

decrease in vitality compared to fresh group ($p<0.05$). This study showed that different doses of hyaluronan didn't have effects on morphology of human sperm.

Conclusion: The present study showed that the effect of hyaluronan on sperm motility and vitality is dose dependant with 1000 µg/ml hyaloronan showing the best results.

O-22: Role of Cyclooxygenases in Male Reproduction

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Arachidonic acid, the major plolyunsaturated fatty acid (PUFA) in cell membrane phospholipids of mammalian systems, is mainly oxygenated by two important pathways, the cyclooxygenase (COX) pathway to generate prostaglandins, thromboxanes and prostacyclin and the lipoxygenase (LOX) pathway to generate leukotrienes and lipoxins. These oxygenated metabolites, collectively termed as eicosanoids, are extremely potent biologically active molecules with bewildering variety of actions. There is no system in the body that is not effected by eicosanoids in one way or the other. In the present study we are working in understanding the role of cyclooxygenases in reproduction. Cyclooxgenase, the rate limiting enzyme in the production of Prostaglandins, exitsts in two isoforms, the constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2).

Even though COX-2 is known broadly as the inducible isoform expressed in response to inflammatory and mitogenic stimuli. We have demonstrated for the fisrs time the consitutive expression of COX-2 in rat testis. The COX-2 mRNA in the testis is smaller (2.8 kb) than that of the inducible COX-2. Hormone treatment (Testosterone/follicle stimulating hormone) regimes increased the levels of COX-2 protein suggesting that sustained levels of COX-2 protein in testis can be influenced by gonadotrophins and androgens. A novel functional association of rat testicular GSTs with cyclooxygenases *in vitro* was demonstrated. These studies reveal a reversible functional interaction between membrane-associated cytosolic glutathione S-transferases (mac GSTs) and COX *in vitro*, with possible interactions between them at GSH binding site. It is proposed that the constitutive COX-2 may be involved in spermaogoneal renewal. Also the role of COX-2, iNOS and oxidative stress in male infertility during endotoxin-induced acute inflammation was elucidated. Inflammation-induced COX-2 was shown to decrease steroidogenic acute regulatory (StAR) protein levels and thus affect testosterone biosynthesis and stermatogenesis in rats.These studies have lead to the basic understanding on the role of constitutive and inducible isoforms of COX-2 in male reproduction.

O-23: Cloning and Sequence Analysis of a Novel Gene Encoding A Testis Lipid Binding Protein (PERF15) in Human Testis

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PERF15 is a testicular germ cell specific fatty acid-binding protein (FABP) isolated from mammals, originally, rat. It encodes for one of the most abundant proteins of the rat spermatozoa localized in perinuclear theca. It is a small protein of 15 060 molecular weight. Northern blot analysis demonstrated that the rat PERF15 mRNA is transcribed in meiotic and post meiotic cells, exclusively. In this study, we have cloned the human PERF15 gene. For this purpose, we have designed two specific primers to amplify the human PERF15 gene according to the open reading frame of automated computational analysis of Homo sapiens similar to testis fatty acid binding protein 9 with accession #XM_378035. After performance the PCR, a unique band of ~3kb was obtained. Restriction digest using PvuII restriction enzyme, confirmed that the fragment is

related to the same gene. Then we gel extracted the ~3kb band. We sequenced this fragment by direct sequencing using Automatic sequencer (version 3130XL Genetic Analyzer, ABI Applied Biosystem) and 2 specific primers to the gene. Alignment showed 100 % similarity between our gene sequence and the mentioned computational data. The human PERF15 gene contains four exons and three introns. Exon 1 codes for 24 amino acids, exon 2 codes for 57 amino acids, exon 3 codes for 34 amino acids and exon 4 codes for 17 amino acids, respectively. The existing three introns are composed of 2113, 461, and 168 nucleotides. In spite of the homology between exonic regions and exon-intron boundaries of the human PERF15 gene and the animal's (rat& mouse PERF15 genes), the human PERF15 gene is different in size and sequence of the corresponding introns with rat and mouse PERF15.

O-24: Increased Expression of Interleukin-1Beta and Interleukin-1β is Associated with Experimental Varicocele

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Objective: To describe the effect of varicocele, in an experimental rat model, on the levels of IL-1β and IL-1 β proteins in testis tissue.
Design: Comparative and controlled study.
Setting: Experimental research.
Animal(s): Wistar male rats in experimental and control groups.
Intervention(s): The control group underwent sham operation (n_6). Experimental groups underwent partial ligation of the renal vein to induce experimental varicocele and were then killed at 9(n_6), 11(n_6), and 13(n_6) weeks after induction of varicocele.
Main Outcome Measures: Histologic evaluation of the varicocele model was determined by periodic acid-Schiff staining of paraffin-embeded testicular tissues. Levels of cytokines were assessed by mmunohistochemistry and Western blot analysis.
Results: Varicocele caused testicular damage, especially in 11- and 13-week-old varicocele groups. In sham-operated rats, Golgi complexes of round spermatids expressed especially the_form of IL-1. By the progression of varicocele, the IL-1_xpression increased temporally in Sertoli cells, spermatogonia, primary spermatocytes, spermatids, and Leydig cells. The expression of IL-1_was seen in Leydig cells in sham-operated rats. The IL-1_ expression was also increased upon progression of varicocele in Leydig cells, Sertoli cells, and spermatogonia.
Conclusion: We suggest that IL-1_ and IL-1_ are the regulators of testicular function. Certain pathologic conditions, e.g., varicocele, cause an increase in the expressions of such proinflammatory cytokines. The increased expression of IL-1_ and IL-1_ in varicocele shifts the balance in favor of inflammatory and immune responses and causes detrimental effects in testis tissue, which may cause male infertility.

O-25: The Effect of LIF on Low Motility Sperms in Men with Asthenospermi

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Introduction: From the past until now extensive study has been taken to deal with the infertility problems in men. Some of the causes are related to men infertility can be defined as reduced number of sperms abnormal morphology and weakness in motility of sperm. Recently improvement in quality of sperm such as number and morphology by in vitro experiments is not possible at the present time. Improvement in quality of sperm motility by means of increasing motility with drugs is possible.

Investigations have shown that closed relation exist between sperm motility and fertility rate by adding progesterone, platelet activating factor, follicular fluid, cytokines & pentoxyphilline by in vitro experimentation which increasing the ability of the sperm motility and survival time. On the other hand, some studies has been done on the effect of LIF on normal sperms motility and shown that this factor could improve normal sperm motility. In this project by knowing the idea that there is no report about the effect of LIF on low motility sperms in men with asthenospermi, we decided to determine effect of this factor on low motility sperms.

Materials and Methods: In this investigation the semen sample of 15 infertile men who have already referred to IVF department of Imam Khomeini Hospital were collected and then preparing them by successive stages for culturing in Ham's F-10 with different concentrations 3, 5, 10, 50ng/ml in incubator on the condition 37 , %5 co2 incubated and samples evaluate for motility and survival time at 6, 24, 48 hours, after culturing, collected data with ANOVA & LSD method by means of SPSS software were analyzed.

Results: The results showed that this factor doesn't effect own motility and survival time of sperm with the duration of 6 hours but after 24 hours of culturing in 10ng/ml and 48 hours of culturing in 50ng/ml concentration of LIF respectively the results show improvement in both motility and vitality of sperm.

Conclusion: Our data showed that LIF has positive effects on motility and vitalaity of human sperm in dose dependant.

O-26: The Philosophy of Testicular Descent

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Mammalian male sex determination is an active process involving complex interactions among several genes. SRY and SOX9 are both responsible for testis formation by initializing and maintaining, respectively, Sertoli cell differentiation. Male sexual differentiation is governed by testicular hormones. Testicular descent (TD) and scrotal evolution occur exclusively but not universally in mammals. Although still debatable, this evolutionarily costly process aims at least to secure lower (than core body) testicular temperatures essential for viable sperm production and storage. TD in scrotal mammals is a multistaged process involving interplay of several anatomical structures and hormonal factors. The gubernaculum appears to play a key role, especially in transabdominal TD (TTD). Androgens and Mullerian inhibiting substance have a rather limited, if any, role during TTD. Leydig cell-derived insulin-like 3-hormone acting directly upon the gubernaculum and proteins encoded by homeobox genes represent good candidate controllers of TTD. Inguinoscrotal TD is mediated by androgens probably acting indirectly upon the gubernaculum, in conjunction with mechanical (abdominal pressure) factors. There is a general agreement on the seasonality of testicular maldescent (TMD) at least in the northern hemisphere. Epididymal malformations, impaired testicular histology due to intrinsic testicular defects, mild hypogonadal state, or increased germ cell apoptotic rate mainly due to abnormal testicular temperature may account for the impaired fertility in individuals with TMD. The theory of an intrinsic testicular pathologic process might plausibly explain the association of TMD with the testicular cancer.

O-27: Experimental Animal Models of Varicocele

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Varicocele models can be induced in rats by partially ligating the left renal vein. Induction of left varicocele in Wistar rat results in a bilateral increase in testicular temperature and a bilateral decrease in epididymal sperm content and motility. The above detrimental effects of left varicocele on bilateral testicular spermatogenesis and epididymal sperm maturation process can be counteracted by the administration of factors inducing

testicular vascularization. The administration of indomethasin or hCG to varicocelized rats improves bilateral testicular function.
Induction of left varicocele in rabbits has shown that there is a defect in glucose metabolism in the varicocelized testis. This experiment tends to suggest that varicocele is a metabolic disease. Further experiments in varicocelized rats or rabbits have shown that left varicocele affects detrimentally the oxidative status of DNA in sperm nucleus. This may be the reason that embryos developed from the fertilization of oocytes with spermatozoa recovered from varicocelized animals have a lower potential for early development and implantation.
Performance of varicocelectomy in experimental animals with left varicocele can ameliorate the detrimental consequences of left varicocele on bilateral spermatogenesis, epididymal sperm maturation, and sperm nucleus oxidative status.

O-28: In Vitro Spermatogenesis

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Induction of meiotic and post-meiotic alterations of male germ cells *in vitro* has been the target of several research efforts since 1960. However, to date, the establishment of an ideal culture system in which spermatogonial stem cells can be maintained and directed to proliferate and undergo meiosis and complete spermiogenesis does not exist. This is attributed to the difficulties concerning the isolation and purification of defined subpopulations of germ cells and the establishment of male germ cell lines. In addition, there is no adequate knowledge regarding the optimal biochemical conditions that promote the survival and differentiation of germ cells in long-term cultures. This lecture focuses on the methodologies that have been proved sufficient to achieve differentiation of cultured male germ cells. Furthermore, the factors regulating spermatogenesis and the technical prerequisites to achieve differentiation of cultured male germ cells will be described. Finally, the role of *in vitro* cultures of immature diploid germ cells in the therapeutic management of men negative for haploid cells in their testes and the subsequent potential genetic and epigenetic risks will be discussed.

O-29: The Effects of Varicocele on Chromatin Condensation and DNA Integrity of Spermatozoa

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Introduction: Varicocele is the most common causes of poor serum production and decreased serum quality.It is demonstrated that patients with varicocele possessed a higher DNA fragmentation index and sperms with nuclear anomalies than healthy fertile men. This may be correlated with an increase of reactive oxygen species in their semen samples. But the effect of varicocele on sperm chromatin condensation is poorly understood. So, the aim of this study is the evaluation of sperm chromatin integrity in these patients.
Materials and Methods: 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. To evaluate sperm chromatin quality and DNA integrity,Aniline blue, Toluidine blue, Chromomycin A3 and finally Acridine orange stainings were done in all of groups.The slides were analyzed and to determine the percentage of mature or immature sperms, 200 spermatozoa were counted in each slide. The data were analyzed by SPSS(13) software and are presented as mean± standard deviation. Statistical significance was set at $p<0.05$.
Results: 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 (pvalue=0.000). There was a significant difference between three groups

with regards to CMA3 results (23.40±6.84, 41.45±10.07, 57.15±8.31 in control, infertile and varicocele groups respectively and pvalue=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(pvalue=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 pvalue=0.000).
Conclusion: 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.

Embryology

O-30: Three Years Experience with Routine Vitrification of Human Pronuclear Stage Oocytes

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Introduction: Cryopreservation of human oocytes and embryos are mandatory tools leading increased cumulative outcome of artifical reproduction techniques while decreasing costs. Vitrification is a cryopreservation technique that leads a glass-like solidification, and rapid freeze of cells and/or tissues. Nowadays it is claimed to be the future of cryopreservation of human embryos due to improved survival rates and clinical outcomes.
This study is conducted at a university clinic to asses the safety and efficiency of vitrification of human zygotes as a routine management.
Materials and Methods: This study consisted of 106 cycles of cryopreserved embryo transfer (cryoET) and vitrification of 2PN stage zygotes of 82 patients who underwent IVF/ICSI at department of gynaecology and obstetrics, University of Luebeck/Germany between March 2004 and July 2007. Depending on the case additional PN zygotes were prepared for vitrification. The PN zygotes (all zygotes of the same patient together for time saving) were incubated in Equilibration solution (ES) comprising 7.5% Ethylene Glycol (EG) and 7.5% Dimethy Sulfoxide (DMSO) in Ham´s F-10 medium supplemented with 20% serum for 8-10 min (according to the time needed for re-expansion of the zygote) at room temperature. After an initial shrinkage and recovery, they were then aspirated and placed into the Vitrification Solution (VS) (15% EG + 15% DMSO+0.5M Sucrose) in Ham´s F-10 medium supplemented with 20% serum for a period not more than 60sec at room temperature. After having observed that Cytoplasmic shrinkage has been taken place, zygotes were aspirated and placed on the tip of the Cryotop. No more than two zygotes were placed on each Cryotop. Cooling of the zygotes was done by direct contact with fresh clean liquid nitrogen (LN2). The Cryotops were capped under the LN2 to seal and protect the vitrified material before cryo-storage for at least two months. Warming of zygotes was performed by placing the Cryotop in Thawing Solution (TS) (1M Sucrose) for a period not more than 60sec. at a temperature of 37 C and then in to Dilution Solution (DS) (0.5M Sucrose) for three min. followed by another DS of 0.25M Sucrose for additional 3 min. at room temperature. The re-warmed zygotes were washed 8-10 times in culture medium before incubation or culture Sage medium under oil for 24 hours prior to embryo transfer. The survival was assessed morphologically at the day of embryo transfer.
Results: The mean age of the patients was 32.18 (standard deviation$\pm$4.47) and the cause of infertility was either male factor infertility or in combination with tubal infertility who were not complicated with hydrosalpinx. A total of 849 PN stage zygotes were vitrified between March 2004 and July 2007. During this era 103 cycles of cryopreserved embryo transfer were completed. Totally 339 PN zygotes were warmed and warming procedure resulted in a 91.15% survival rate (309 PN zygotes). A three times higher pregnancy rate (36.8%) was obtained when compared to the results of slow rate freezing method (10.2%) which was previously used for a long time in the same centre. In conclusion vitrification of

human zygotes at PN stage seems to be a successful and reliable method with favourable outcomes which can be recommended as a routine technique of cryopreserved of human embryos.

Conclusion: Routine vitrification of human zygotes is an efficient and reliable method of cryopreservation of zygotes and seems to improve clinical outcomes of artificial reproductive techniques.

O-31: Apoptosis in Mouse Embryos Co-Cultured with Polarized or Non-Polarized Uterine Epithelial Cells Using Sequential Culture Media

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Introduction: This study investigated the effects of the in vitro co-culture of mouse embryos with non-polarized or polarized uterine epithelial cells, using sequential culture media, on their development to blastocysts, blastocyst quality (blastocyst diameter and cell number) ,the onset and frequency of apoptosis as well as morphological changes that confirm to the general criteria of apoptosis by using a terminal deoxy nucleaotidyl transferase-mediated dUDP nike-end labeling(TUNEL) assay and expression of apoptotic related gene including Bcl-2 and Bax using RT-PCR.

Materials and Methods: There were three treatments, all of which used sequential culture media. The treatments were no co-culture (control), non-polarized or polarized epithelial cell monolayer co-culture in 24-well tissue culture plates. Mouse uterine epithelial cells were isolated enzymatically and were seeded either on the surface of the culture plate (non-polarized monolayer) or on a Millipore filter insert coated with extra-cellular matrix extract (polarized monolayer) that was then placed in the culture plate. Two-cell mouse embryos were cultured in G-1^{TM}ver3 medium to the 8-cell stage when they were randomly assigned to the treatments. The culture medium was G-2^{TM}ver3 during the treatment phase of the study. Significances of differences were evaluated by the one way analysis of variance for continuous data.

Results: The epithelial cells cultured on Millipore filters became polarized and their morphology compared favorably with those cultured on the surface of the culture plate and in vivo uterine epithelial cells. After 96 h on the treatments, the polarized monolayer had supported the development of significantly more hatched blastocysts (80.0%; $p<0.05$) than the non-polarized monolayer (63.4%) or the control (61.4%) culture treatments. Co-culture resulted in the production of blastocysts with significantly more cells (non-polarized monolayer 56.7±2.1, polarized monolayer 61.9±2.1) than the control culture (42.8±2.6; $p<0.05$) but the diameter and shape of the blastocysts were not significantly different. The proportion of blastocysts with apoptotic blastomere was higher for the control culture (94.4%) than for the non-polarized (68.2%) or polarized (66.7%) co-culture systems ($p<0.05$).

Moreover, the apoptotic index was significantly higher in control blastocysts (5.6±0.9; $p<0.05$) than in non-polarized (1.7±0.3) or polarized (1.5±0.3) co-culture. In the control, Bax mRNA was strongly expressed when compared to co-culture treatments ($p<0.05$), whereas, the relative abundance of Bcl-2 mRNA to the β-tubulin was lower than co-culture treatments ($p<0.05$).

Conclusion: It is concluded that a co-culture system especially involving polarized uterine epithelial cells and sequential culture media improved mouse embryo development by decreasing the incidence of apoptosis and it is a promising method of producing mouse embryos.

O-32: Extract of Azadirachta Indica (Neem) Leaf Induces Apoptosis in Rat Oocytes Cultured *In Vitro*

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Objective: To determine whether aqueous neem leaf extract (NLE) could induce degeneration of rat oocytes. If yes, whether apoptosis is involved during NLE -induced degeneration of oocytes cultured in vitro.
Design: A controlled prospective study.
Setting: Laboratory research setting at Department of Reproductive Biomedicine of the Institute.
Animal(s): Fifty four sexually immature female rats that were 24-25 days of age.
Intervention(s): The immature female rats were injected with 10 IU pregnant mares serum gonadotropin (PMS) for 48 hrs followed by 10 IU of hCG for 16 hrs. After 16 hrs, rats were euthanized; ovulated cumulus oocyte complexes (COCs) were collected from oviduct. Cumulus-enclosed as well as denuded oocytes were used in the present study.
Main outcome Measure(s): Rates of shrinkage, membrane leakage, degeneration, assessment of morphological apoptotic changes, bax protein expression and DNA fragmentation.
Results: The NLE induced morphological apoptotic changes such as shrinkage, membrane leakage, cytoplasmic fragmentation prior to degeneration of oocytes. The NLE-treated oocytes that had morphological apoptotic features showed overexpression of bax protein, DNA fragmentation as evidenced by TUNEL positive staining and DNA ladder pattern.
Conclusion: NLE-induced apoptosis in rat oocytes prior to degeneration in vitro.

O-33: Ovarian Tissue Transplantation: A new Method and Site for Induction of Folliculogenesis in Mice as a Model for Human Female

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Introduction: Ovarian tissue transplantation is a new method of restoring fertility to women whose ovaries are not functioning normally. Young women who undergo chemotherapy or radiotherapy for cancer face serious consequences to their reproductive health and severely affect the ovarian follicular store, especially. Therefore, the aim of this study was to demonstrate induction of the folliculogenesis from ovarian tissue (OT) transplanted under kidney capsule in the presence or absence of gonadotropins support.
Materials and Methods: Forty eight healthy female mice were anesthetized and abdominal cavity is open. From one side of the body, small piece (~1 X 1 X 1 mm) of OT was transplanted to the subcapsular membrane of kidney at another side, and surgical operation is closed. Then, female mice were classified into three groups according to the time of gonadotropins injection. Group-1: mice injected with sterile normal saline (control group). Group-2: mice injected with gonadotropins directly for four days. Group-3: mice injected with gonadotropins for four days after eight days of surgical operation. Follicular growth, quality of retrieved ova and histological changes for transplanted OT were assessed.
Results: In general, no deletion for transplanted OT pieces and no side effects post-operation on mice of all groups were recorded. Best follicular growth of transplanted OT was achieved for groups 1 and 2. Graafian follicles were obtained from transplanted OT of group-2, and less degree for group-1. However, least degree for follicular growth of transplanted OT was reported for group-3 as compared to other groups. Immature and mature oocytes with corona and cumulus cells collected by squashing of transplanted OT.
Conclusion: The present data demonstrate that the ovarian tissue transplantation is possible to undergo follicular growth subcapsular of the kidney. Also, physiology of the body supports the ovarian follicular growth in another site other than normal position. Further studies are recommended on in vitro

maturation and fertilization of retrieved ova and embryo transfer.

O-34: The Effect of Necrotic Blastomere Removal on Vitrified – Warmed 4-Cell Stage Mouse Embryos Development

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Introduction: 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.
Materials and Methods: The retrieved 2-cell mouse embryos were cultured in G1TM ver3 medium until the 4-cell stage, then the embryos were vitrified with pretreatment and vitrification solutions. After warming the embryos were transferred to G1TM 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 G2TM ver3 medium. Finally, the rate of blastocyst formation, number of total blastomeres, 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.
Results: 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.
Conclusion: The necrotic blastomere removal improved development and quality of partially damaged vitrified – warmed mouse embryos.

O-35: Cryopreservation of Spermatozoa in Alginic Acid Capsules

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Objective: To develop a method of freezing small amounts of spermatozoa in polymerized alginic acid drops, which can be liquified after thawing for recovery of the spermatozoa.
Design: Prospective clinical study.
Setting: Medical School, RWTH Aachen, Aachen Germany.
Patient(s): None.
Intervention(s): Validation of the encapsulation method with bovine sperm; cryopreservation of human spermatozoa in alginic capsules.
Main Outcome Measure(s): We optimized the cryopreservation method by testing different parameters influencing the freezing procedure, such as concentration of alginic acid, size of drops, time of polymerization, and culture media.
Result(s): The final protocol was as follows: encapsulation by 7.3 mg/mL alginic acid forming 10-_L drops polymerized for 30 seconds and liquefied for 2.5 minutes in sodium citrate. Cryopreservation of human spermatozoa by this protocol resulted in a decreased motility of 18.3% compared with standard protocols but a 19.9% higher vitality of the immotile spermatozoa.
Conclusion(s): No difference in viability of spermatozoa after both sperm-freezing procedures could be observed.
Further investigation will be undertaken to reduce the amount of immotile but viable sperm after microencapsulation in alginic acid.

O-36: Assisted Oocyte Activation Using Ionomycin does not Lead to

Subsequent Normal Calcium Oscillation Pattern

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Introduction: At fertilization sperm triggers oscillations in the cytoplasmic calcium concentration ([Ca^{+2}]i), that are necessary for oocyte activation and normal further embryo development. Artificially raising [Ca^{+2}]i in oocytes injected with round-headed sperm using a Ca^{+2} ionophore is however sufficient to achieve normal fertilization and embryo development as evidenced by successful human pregnancies and births. However, it is not known yet if assisted oocyte activation can induce the calcium oscillations normally observed after fertilization.
Materials and Methods: Mature mouse oocytes collected were loaded with fluo3-AM and injected with round-headed sperm from the consenting patient or sperm from a consenting proven fertile man. Thirty minutes after injection oocytes injected with round-headed sperm were incubated with the Ca2+ ionophore ionomycin (10µM) for 10 minutes. Changes of fluorescent intensity were recorded immediately after treatment. Extrusion of the second polar body (PB2) and two-cell formation were assessed.
Results: None of the oocytes injected with round-headed sperm were activated (0/12) and none of them displayed [Ca2+]i changes. After treatment with ionomycin more than half (42/59) of the round-headed sperm injected eggs were activated and extruded PB2 2 hours later. Ionomycin treatment was associated with a transient [Ca^{+2}]i rise but was not followed by any oscillatory activity over the recording period. By contrast, in almost all of the 19/38 oocytes injected with fertile sperm that extruded PB2, [Ca^{+2}]i oscillations were observed.
Conclusion: A single transient rise in [Ca^{+2}]i triggered by ionophore treatment appears to be sufficient to bring about activation of round-headed sperm injected oocytes, but this does not lead to subsequent [Ca^{+2}]i oscillations. Successful clinical application in the human, however, suggests that this does not seem to prevent the further embryonic development. These experiments thus bring up the question on the role of [Ca^{+2}]i oscillations on embryo development after ICSI with round-headed sperm.

O-37: A Morphometric Study on the Endometrium of Rat Uterus in Hypothyroid and Thyroxine Treated Hypothyroid Rats

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Hypothyroidism increases the rate of pregnancy loss. Other manifestations include menstrual disorder, and infertility. Serum levels of gonadotropins are low in hypothyroid patients. Though studies of uterine ultrastructure are well established as approaches to investigating the pathophysiology of infertility, they have scarcely been extended to the study of hypothyroid related infertility. The present study investigates the effect of hypothyroidism on the ultrastructure of uterine epithelium.
Three groups of Wistar rats were studied. Two groups were initially made hypothyroid using methimazole, and the third group was an untreated control. One hypothyroid group was given daily injections of thyroxine for six weeks. The uteri were removed in all three groups, and processed for transmission electron microscopy and morphometry. It was found that absolute epithelial cell volume was decreased in hypothyroidism. The volume of the nucleus had decreased though its relative volume in the cell had

increased. The height of the luminal epithelium in hypothyroid rats also decreased by (33.8%) as compared with controls. Basement membrane thickness was significantly increased in hypothyroidism. The changes were all substantially abrogated by the administration of thyroxine.
This study suggests that thyroid hormones might be importantly concerned in the maintenance of the normal structure of uterine epithelial cells.

O-38: History and Fundamentals of Oocyte Maturation in Vitro

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Oocyte maturation is generally defined as the reinitiation of the first meiotic division leading to metaphase II (MII), combined with the appropriate cytoplasmic processes, which are necessary for proper fertilization and early embryo development. Using in vitro maturation techniques, oocytes are harvested in the GV stage (germinal vesicle, prophase I), and matured in special maturation media.
Relevant aspects of oogenesis and follicular development will be described.
In vitro maturation (IVM) of oocytes was first carried out in rabbits in 1935 by Pincus and Enzmann followed by Robert Edwards, who in 1965 did the first IVM on human oocytes. Ironically, the first IVM baby was the result of *in vitro* maturation of an immature oocyte from a stimulated cycle (Veeck 1983). Starting with Cha in 1989 the number of babies resulting from *in vitro* maturation of oocytes in unstimulated cycles has steadily increased.
Several reasons for using IVM can be mentioned and will be discussed in this lecture, but especially two major groups of patients are interesting for this procedure:
One group consists of regularly cycling women with normal ovaries referred for IVF and ICSI due to severe male infertility problems or tubal factor.
The other group consists of women suffering from PCO/PCOS. These women are extremely sensitive to stimulation with follicle stimulating hormone (FSH) and have a significant risk of developing ovarian hyperstimulation syndrome (OHSS). Collection of immature oocytes combined with *in vitro* maturation may eliminate the risk of OHSS.
The IVM techniques are still being developed and improved, and were most recently discussed at the Second International Symposium on *In Vitro* Maturation of Oocytes in connection with the ESHRE Meeting in Lyon, France, on July 1 2007. At this meeting an attempt was also made to produce an educated estimate of the number of IVM deliveries and ongoing pregnancies. This estimate ended up being around 1150 until the middle of 2007. It should be mentioned that more than half of those are from Asian countries.

O-39: Clinical in Vitro Maturation of Human Oocytes

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Oocyte maturation is generally defined as the reinitiation of the first meiotic division leading to metaphase II (MII), combined with the appropriate cytoplasmic processes, which are necessary for proper fertilization and early embryo development. Using *in vitro* maturation (IVM) techniques, oocytes are harvested in the GV stage (germinal vesicle, prophase I), and matured in special maturation media. Especially two major groups of patients may benefit from IVM:
One group consists of regularly cycling women with normal ovaries referred for IVF and ICSI due to severe male infertility problems or tubal factor.
The other group consists of women suffering from PCO/PCOS. These women are extremely sensitive to stimulation with follicle stimulating hormone (FSH) and have a significant risk of developing ovarian hyperstimulation syndrome (OHSS).
An IVM expert meeting in Copenhagen in autumn 2006 came up with recommendations for the treatment of these two groups of

patients:
Inclusion criteria:
Age ≤ 35 years
BMI 18 – 25 kg/m^2
≤ 3 previously failed IVF cycles
≤ 3 previous IVM attempts
No endocrine abnormalities (e.g. hyperprolactinemia, hyperglycaemia, hyperthyroidism)
No ovarian cyst>20 mm on cycle day 3
Estradiol level<0.2 nmol/L or 70 pg/ml on cycle day 3
Endometrium < 4 mm and fully exfoliated on cycle day 3
FSH<12 IU/L on cycle day 3 *(only PCO/PCOS)*
≥5 antral follicles of 2-5 mm on cycle day 3 *(only regular cycling patients)*
Normal ovulatory cycles (26-35 days) *(only regular cycling patients)*
Priming:
FSH: *PCO/PCOS patients:* RecFSH (150 IU) is administered on cycle day 3, 4 and 5 *Regular cycling patients:* No FSH
hCG: No hCG is administered 17-β-Estradiol: From day of oocyte pick-up (Day-1). 2 mg orally three times daily.
If pregnancy test is positive continue at least until 50 days gestation, otherwise stop.
Progesteron: Starting 2 days after OPU (Day 1). 600 mg vaginally daily. If pregnancy test is positive continue until gestation week 10 - 12, otherwise stop.
Oocyte pick-up (OPU) scheduled when:
The leading follicle is 12 mm (10-14 mm)
If the leading follicle is larger than 14 mm, convert to IVF with hCG administration *(only regular cycling patients)*
The endometrium is at least 5 mm thick
Oocytes are collected from small antral follicles, 2-10 mm.
Needle: Single lumen (size not crucial 17-20G)
or IVFJapan needle (19/17 G)
Pressure: 80 – 100 mmHg (7.5-8.0 kPa)
Embryo transfer:
Embryo transfer takes place on Day 2, 3 or 5 -similar to standard IVF procedure. Some IVM centers choose to evaluate the endometrium on the day of planned transfer. In order to optimize synchrony of embryo development and endometrial stage, these groups may choose to freeze the embryos for later transfer.
Media:
MediCult Flushing Media (preferably with Heparin)
MediCult LAG Medium
MediCult IVM® Medium
MediCult ISM1 Medium, EmbryoAssist Medium or Universal IVF Medium
In case of blastocyst transfer also BlastAssist Medium or ISM2 Medium (combined with UTM Medium).
Collection and identification of oocytes:
Oocytes are collected from the follicular fluid preferably using a strainer.
The strainer and the oocytes are washed with Flushing Medium. Atretic and nude oocytes are discarded, while oocyte-cumulus complexes are transferred to LAG Medium for 2-3 hours, then transferred to the equilibrated Maturation Medium.
Components of the Maturation Medium:
MediCult IVM® Medium (refrigerated)
FSH stock solution (frozen)
hCG stock solution (frozen)
Patient serum (fresh)
Check for maturation (Day 0):
After 28-30 hours' incubation in the maturation medium the oocytes are checked for maturation.
Mature oocytes are transferred to MediCult Universal IVF Medium or EmbryoAssist Medium for insemination by ICSI or IVF. Further culture of the embryos take place similar to standard IVF procedures.
The IVM clinical and laboratory techniques will be discussed.
The advantages and drawbacks of IVM will be discussed. The generally lower pregnancy rate per OPU, as well as per ET in IVM cases is largely due to the lower number of oocytes and embryos which lead to a lower transfer rate and a lack of selection possibilities. Improvements of IVM techniques should therefore focus on increasing the number and the quality of oocytes and embryos.

O-40: Letrozole Effect on *In Vitro* Culture of Human Endometrial Explants in Fibrin Matrix

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Introduction: In vitro culture of human endometrial tissue introduced as a model for endometriosis. Drug treatment options in endometriosis are unsatisfactory and more researches on this disease are needed. Aromatase inhibitors are candidate drug in the treatment of endometriosis. The aim of the present study is to investigate an aromatase inhibitor (letrozole) effects on human endometrium in three-dimensional (3D) fibrin matrix.
Materials and Methods: Normal human endometrial biopsies (N=8) from reproductive age women (mean age 28.3 year) were cultured in fibrin matrix for 21 days as a control and different doses of letrozole (0.1, 1 and 10 µM). Tissue changes were evaluated by two systems: an invert microscopic grading system (Marking criteria) and computerized program analysis. Routine histochemical and immunohistochemichal staining were done to document stromal, epithelial and endothelial cells. Data were analyzed by one way ANOVA and post hoc Tukey test.
Results: Letrozole (0.1, 1 and 10 µM) exert a significant ($p<0.001$) dose dependent increasing growth effect on endometrial tissue in this tissue culture model.
Conclusion: Letrozole improve growth of normal human endometrium. This new finding should consider in using this drug.

O-41: Neither Aurora B Activity nor Histone H3 Phosphorylation Is Essential for Chromosome Condensation during Meiotic Maturation of Porcine Oocytes

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Aurora kinase B (AURK B) is a chromosomal passenger protein that is essential for a number of processes during mitosis. Its activity is regulated by association with two other passenger proteins, INCENP and Survivin, and by phosphorylation on Thr 232.

In this study, we have examined expression and phosphorylation (Thr-232) of Aurora kinase B during meiotic maturation of pig oocytes in correlation with histone H3 phosphorylation and chromosome condensation. We show that histone H3 phosphorylation on Ser-10, but not on Ser-28, correlates with progressive chromosome condensation during oocyte maturation; Ser-10 phosphorylation starts around the time of the breakdown of the nuclear envelope, with the maximal activity in metaphase I, while Ser-28 phosphorylation does not significantly change in maturing oocytes.
Treatment of oocytes with 50 uM Butyrolactone I (BL-I), an inhibitor of cyclin-dependent kinases, or cycloheximide (10ug/ml), inhibitor of proteosynthesis, results in a block of oocytes in the germinal vesicle (GV) stage, when nuclear membrane remains intact, however, condensed chromosome fibers or highly condensed chromosome bivalents can be seen in the nucleoplasm of BL-I- or cycloheximide-treated oocytes, respectively. In such treated oocytes no or only a very weak activity of Aurora kinase B, as well as phosphorylation of histone H3 on Ser-10 can be detected after 27 hours of treatment, whereas phosphorylation on Ser-28 is not influenced. These results suggest that Aurora kinase B activity and Ser-10 phosphorylation of histone H3 are not required for chromosome condensation in pig oocytes, but might be required for further processing of chromosomes during meiosis.

O-42: Nuclear Donor Choice, Sperm-Mediated Activation and Embryo Aggregation: A Multipronged Approach to Sequentially Improve Cattle Cloning Efficiency

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Cloning by somatic cell nuclear transfer (SCNT) remains very inefficient with only

about 1-5% of cloned blast cysts developing into viable offspring. In order to improve cloning efficiency, I first developed a zona-free NT procedure which doubles the throughput in cloned embryo and offspring production in cattle and mouse, increasing both ease of operation and reproducibility. I then used this method to determine at which step the NT procedure could be improved to increase cloning efficiency. I focused on the choice of nuclear donor cell type and cell cycle stage, the artificial activation method and cloned embryo culture conditions. Firstly, I hypothesized that cloning efficiency is inversely correlated with donor cell differentiation status and could be increased by using undifferentiated somatic stem cells as donors. By cloning the world,s first red deer from multipotent antler stem cells and their differentiated progeny, we showed that this was not the case. This finding was confirmed in cattle where myogenic cells of divergent differentiation status resulted in very similar cattle cloning efficiency, suggesting that cell plasticity and epigenetic reprogramming are biologically unrelated and somatic donor cell type is not critical for cloning success.we further demonstrated that, independent of donor cell type and cll line, cloning efficiency can be more than doubled by simple serum-starvation of donor cells. The next step towards improving cloning efficiency was using sperm rather than artificial means to activate cloned embryos. Implantation and birth of live offspring were significantly improved after sperm- mediated activation. Finally, we aggregated individual cloned embryos during in vitro culture to further increase cloning efficiency. In embryonic clones, aggregation led to a2-3 fold significant increase in survival to term. Somatic clones, however, showed compromised in vivo survival, revealing striking biological differences between embryonic and somatic clones in response to aggregation.

O-43: GSH Level, Spindle Area and Rate of IVF in Presence of Antioxidant in Mouse Oocyte Cultured in MEME Medium

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Introduction: The study was carried out effects of different doses of cysteamine on rate of IVM and IVF. Also the study was carried out to study glutathione (GSH) synthesis in presence of cysteamine without cumulus cells in MEME medium. MII spindle area was analysed by immunocytochemistry for quantification of shape and size of it.
Materials and Methods: Female mice were primed with 5 IU of PMSG and GV oocytes were retrieved from the ovary 48 hr later for IVM. The IVM medium was supplemented with 0, 50, 100, 200 and 500 mM of cysteamine. Experiments also included a group of ovulated oocytes (in vivo matured) after priming with PMSG and HCG. Cytoplasmic GSH level was measured by DTNB-GR recycling protocol. For IVF MII oocytes were inseminated with mature mouse sperm and 24 hours after insemination rate of two cell embryo was measured. MII Oocytes were fixed and immunostained for microtubules, and chromosomes and then spindle area were analysed.
Results: After IVM, an improvement was observed on MII development in 200 µm cysteamine group. Intracytoplasmic GSH level increased in presence of cysteamine in 200µm cysteamine and Highest level of GSH was produced in In vivo. Spindle area in all in vitro groups exept 500µm increased and Spindle area in 200µm cysteamine compare to in vivo group was insignificant($p>0.05$).
Conclusion: Our results showed that cysteamine improved IVM rate in dose dependant. Rate of two cell embryo increased significantly in 200µm cysteamine compare to control. Also cysteamine induced glutathione synthesis in MII oocyte and improved microtubule organization in 200µm cysteamine group.

O-44: Mouse Back Muscle as a Promising Site for Human Ovarian Tissue Xenotransplantation

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Introduction: Loss of follicles during initial ischemia constitutes the main limitation of ovarian tissue transplantation (OTT). We investigate the efficiency of the back muscle (B) site for human OTT using a SCID mouse xenografting model.
Materials and Methods: 1) Study of vascularization and integration of OT in the grafting site during the first eight days after transplantation and Immunohistochemical staining (Anti-human and anti-mouse CD31 and CD34) to evaluate neovascularization. 2) Follicular development in B and K xenografts, three, five and seven months after grafting.
Results: 1) Anti-mouse CD34 and CD31 positive endothelial cells were first noticed in B and K grafts on days 3 and 4 respectively. On day 5 all B and 70% of K grafts and on day 8, all B and K grafts were positive for mouse markers, but a higher number of murine blood vessels were counted in the B versus K grafts (14.0±1.3 versus 6.60±0.68, $p<0.01$). All ovarian fragments were positive for anti-human CD31 and CD34 antibody, although from day seven onwards a non-significant decrease in blood vessel density was observed. B grafts were completely integrated within surrounding muscle tissue compared to K grafts. 2) Antral follicles up to 15mm were obtained from the B while for the K the largest follicle was only 3.4 mm.
Conclusion: Our results show that murine neovascularization happens faster and to a larger extent in B than K. This suggests that ischemia after transplantation to the B site can be prevented by fast support of murine blood vessels.
B allowed larger follicle size development than K. As far as we know this is the first report of a 15 mm human antral follicle obtained after xenografting to a B site.
In a human xenograft model the back muscle tissue site is promising for further exploration as a model for human OTT.

O-45: Trophoblast Cell Activation by Trophinin Ligation is Implicated in Human Embryo Implantation

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During human embryo implantation, trophectoderm mediates adhesion of the blastocyst to the uterine epithelium. The rapid growth of the embryo and invasion of the maternal tissue suggest adhesion-induced activation of the embryonal cells. We show here that ligation of trophinin, a homophilic cell adhesion molecule expressed on trophoblastic cells, induces tyrosine phosphorylation in trophinin-expressing trophoblastic HT-H cells. The phosphorylation could be induced in HT-H cells with the binding of trophinin-expressing cells or anti trophinin antibodies. Trophinin-dependent tyrosine phosphorylation was associated with actin reorganization. We also isolated trophinin-binding peptides from phage libraries. These peptides exhibited the consensus sequence GWRQ and seemed to reproduce the effects of trophinin-mediated cell adhesion. Upon binding of a GWRQ peptide, HT-H cells became highly proliferative and motile. HT-H cells expressed ErbB family receptors and bound EGF and heparin-binding EGF-like growth factor (HB-EGF), but ErbB family receptor phosphorylation in these cells required

GWRQ. In the absence of GWRQ, trophinin interacted with the cytoplasmic protein bystin, which binds to ErbB4 and blocks its autophosphorylation. In HT-H cells, GWRQ peptide dissociated trophinin from bystin, and ErbB4 was activated. Culturing monkey blastocysts in the presence of the peptide increased total number and motility of the trophectoderm cells. These results suggest that trophinin-mediated cell adhesion functions as a molecular switch for trophectoderm activation in human embryo implantation.

O-46: Evaluation of Reproductive Potential after Intracytoplasmic Sperm Injection of Varied Human Semen Tested by Antiacrosomal Antibodies

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Objective: To determine whether varied human spermatozoa detected also with monoclonal antibodies against acrosomal proteins, have an influence on fertilization, transfer, pregnancy and implantation rates using ICSI.
Design: A retrospective study.
Setting: A private IVF center and academic research laboratory.
Patients: 1240 men participating in the ICSI program.
Interventions: Sperm were divided into seven groups: oligozoospermia, oligoasthenozoospermia, ligoasthenoteratozoospermia, fresh and frozen/thawed epididymal and fresh and frozen/thawed testicular sperm. Fertilization, transfer, pregnancy and implantation rates were recorded in each category. Sperm were tested with antibodies for detection of the of the sperm acrosome.
Main outcome measure: Fertilization, transfer, pregnancy and implantation rates, percentage of acrosome-reacted cells.
Results: The fertilization rate and statistical evaluation showed differences between morphologically normal and pathological sperm and other groups. The freezing-thawing procedure had no influence on the fertilization of testicular sperm but epididymal frozen/thawed sperm had a higher fertilization rate. Immunofluorescence proved decreasing sperm quality in all groups compared to the control group. This difference is not manifested in other parameters (transfer, pregnancy, implantation rates).
Conclusion(s): The spermatozoa with varied semen characteristics and good quality, also detected with specific antibodies, gave the best fertilization rates. The paternal effect is not proved in other parameters.

O-47: Correlation of Somatic Cell Steroid Secretion and Quality of Generated Oocytes after *In-Vitro* Stimulation of Mouse Follicles

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During folliculogenesis, follicular cells are crucial in initiating oocyte development and providing with nutrients and growth regulators for the oocytes. Somatic follicular cell differentiation is well coordinated with oocyte maturation. The objective of this project is to test if follicular cell steroidogenesis can be used as a marker for the quality of the embrace oocytes. We have developed an in-vitro culture system that supports the growth of preantral follicles and retains their competency for fertilization and subsequent embryo development in mouse models. Mechanically isolated mouse preantral follicles were cultivated singly in microdroplets under oil in medium supplemented with recombinant FSH and LH at 37°C and 5% CO2.Under an optimal concentration of FSH and LH, these follicles underwent dramatic morphological changes, which ultimately led to the formation of antral follicles and the production of oocytes. At the initial stage of in-vitro culture (IVC)/maturation (IVM) of follicles, a high level of LH or FSH in the medium

facilitates E2 sevretion, enhances granulosa cell (GC) outgrowth, consequently leading to earlier antral formation. However, prolonged culture in high LH and FSH triggers early differentiation and luteinization of GCs,
resulting in fewer metaphase II oocytes and blastocysts. Under the optimal concentration of LH (10mIU/ml) and FSH (100mIU/ml), follicular E2 production associated with matured oocytes was significantly higher than that of immature ones. Furthermore, matured follicles producing E2 with the range of 60-80ng/ml produced oocytes of highest quality. Approximately 89% of MII oocytes showed an optimal level of E2 prior to ovulation and all of them were able to be fertilized and develop into blastocysts; wheras those oocytes producing a undesirable level of E2 degenerated progressively. In summary, active somatic cell steroidogenesis prior to ovulation and an ideal steroid milieu at ovulation are critical for the gereration of competent oocytes after follicular maturation invitro.

O-48: ART Lab Quality Control

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O-49: Blastocyst Culture and Transfer-What Are the Essentials to be Successful

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Epidemiology and Ethics

O-50: The Beginning of Human Life and the Embryos: A Philosophical and Theological Perspective

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Life is a process which has a beginning and an end. Every health care professional, especially the one who "plays" more at these edges, should have a very clear position on when a human life begins and ends. This is very important, since the moral acceptability of some medical applications depends on the definition of these times. There are different views from the scholars in different academic fields on the time of the beginning of human life. Although there is not much debate on the "humanity" of a newborn, except some marginal philosophers, the moral status of the embryos and fetuses are still debated.
In this presentation, it will be discussed whether determining the beginning and the end of life is a matter of moral or medical decisions. As it will immerge from the discussion that, it is a matter of moral decisions, the concept of human life will be defined from social sciences perspective, and the time of its beginning will try to be explored. The presentation will show that human life begins, therefore morally matters, in the womb at the beginning of 8th week after conception. After referring some bioethical concepts, like 'human being', 'human person' and 'moral being', all these conclusions will be applied to Assisted Human Reproduction, genetic cloning and stem cell research. Finally, under the light of these information, applicability of these techniques will be evaluated from cultural and faith tradition perspective.

O-51: Using Embryonic Stem Cells in Therapeutic Research: A Theological Perspective

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Stem cell research is a recent technology that has been discussed by the scholars from various disciplines since it has different

dimensions that relates to these disciplines. Although the scientists in the field of biotechnology argues that this technology will be used for the benefit of the society it requires the legal, ethical, and scientific issues associated with this research be critically addressed and articulated. There is a necessity to distinguish two different types of stem cells, namely adult and embryonic origin. It is generally believed that it is morally less problematic to use adult stem cells, whereas there are some concerns, especially from theological perspective, to use embryonic stem cells in reproductive and therapeutic cloning.
In this presentation we aim to address to the usage of embryonic stem cells for therapeutic purposes, and will argue that it is not only allowable to use embryonic stem cells for this purposes but also there are moral imperatives to use them since there is not enough concrete evidence to justify discontinuing the research on stem cell research. We discuss the issue from a theological perspective with special reference to Islam.

O-52: Ethical Aspects of Regulation of Assisted Reproduction in Arab Speaking World

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O-53: Stress and Infertility and Using Stress Management Techniques

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O-54: Social Sex Selection and the Balance of the Sexes: Empirical Evidence from Germany, the UK and the US

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Introduction: Preconception sex selection for nonmedical reasons is one of the most controversial issues in bioethics today. The most powerful objection to social sex selection is based on the assumption that it may severely distort the natural sex ratio and lead to a socially disruptive imbalance of the sexes.
Materials and Methods: Based on representative social surveys conducted in Germany, the United Kingdom, and the United States, this paper argues that the fear of an impending sex ratio distortion is unfounded.
Conclusion: Given the predominant preference for a "gender balanced family", a widely available service for social sex selection is highly unlikely to upset the balance of the sexes in Western societies.

55. The Future (r)Evolution of PGD/HLA- Testing; Ethical Reflections

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56. PGD of Hereditary Cancers: Ethical Aspects

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O-57: Human Cloning and Human Dignity: Catholic and Islamic Perspectives

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O-58: Attitudes of Turkish and Iranian People Towards Egg Donation

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Introduction: We conducted two seperate descriptive studies in order to reveal the General attitudes of Turkish and Iranian people toward various aspects of oocyte donation (OD).
Materials and Methods: The first Study was carried out in two seperate districts of the city of Antalya, Turkey. 400 particioants were chosen by dluster sampling method. The questionnaires were performed by 4th year medical students face to face with the participants. The second study was conducted in Isfahan city, Iran. Two hundred adults (Christians, n=100; Muslims, n=100) were asked to fill out the questionnaires.
Results: The Turkish participants consisted of 232 women and 168 men. 64.75% were married, 5% were divorced. 63.75% had children, 15(3.75%) had infertility problems, 263(65.75)% were graduates of high school or university. Approval of egg donation was high in this study sample. Only 61 (15.25%) respondents showed complete objection againts egg donation and more men were in favor. Less than half of the participants think that their religion would prevent egg donation if they need it. More than half of the participants would prefer the use of egg donation treatment rather than adopting a child.
For the Iranian participants, 53% of Christians and 69% of Muslims were married. The vast majority of the subjects had formal education; only 3 of the participants were illiterate. Only one-third had complete objection toward OD program. 68% and 43% of the Christians and Muslims were unaware of their religious attitudes on OD, respectively. Most of participants believed in informing the general public about OD in mass media. In addition, nearly half of the participants were in favor of OD over adopting a child. Psychological counseling was recommended by majority of respondents for both donor and the recipient of the eggs.
Discussion: These two studies reveal the first data on the attitudes towards OD from countries whose population is mainly muslim. The most important conclusion is the fact that most of the participants do not have any objection against egg donation treatments. However, the vast majority did not know for whom the OD program is suitable. Therefore, the mass media should develop programs for informing the general public regarding the OD treatment program.

O-59: Stem Cell Research: Ethical and Religious Issues

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Introduction: Stem cells are undifferentiated, primitive cells with the unrivalled ability to differentiate into any specialized cell type. The discovery of stem cells early in the 1980s has promised new treatments and possible cures for many debilitating diseases and injuries. Research using embryonic stem cells (ESCs) is a very important area of current biomedical investigation. Iran is one of the first countries which have produced human embryonic stem cells. However, stem cell research have raised a series of ethical and religious questions that are being confronted by multiple international organizations, nations, cultures, and religious traditions. Our aim is to review the main ethical issues in this challengeable field, considering the different religious viewpoints. The national specific guideline for the gamete and embryo research (2005) will be stated.
Materials and Methods: For compiling the article, we have searched articles in Google and Ovid search engines, PubMed, and IranMedex sources by using appropriate

keywords. We have also used the Holy Koran and religious opinions from great Muslim scholars. We have referred to some English, Arabic and Farsi books in this field.

Results: Stem cells can be obtained from different sources. Using adult stem cells is relatively free of ethical conflicts. But embryonic stem cell (ESC) research is controversial because harvesting the stem cells destroys the embryo. The status of the pre-implantation embryo is the most sensitive and disputed point in the debate on isolation of human ESCs for research. Considerable differences of opinion exist with regard to the ontological and moral status of the pre-implantation embryo. On the other hand, many people believe that permitting this type of research paves the way for reproduction of an entire human and will open the way to a slippery slope of dehumanizing practices, such as embryo farms, cloned babies, the use of foetuses for spare parts, and the commodification of human life. The issue of "Proportionality", "Justice", "Resource Allocation" and "Subsidiarity" are a number of other important debates in this field.

There is no consensus on the morality of the embryo, even within particular religious traditions. In Islam the embryo, even in the first day of its existence, has the right of life but according to some decrees (Fatwa), the use of embryo for therapeutic or research purposes may be acceptable under necessity if that is takes place before the point at which the embryo is ensouled.

Conclusion: Research involving human embryos could be permitted for therapeutic purposes with full considerations and all precautions. Cooperation of scientists, ethicists, jurisprudents and lawyers is essential for establishing a culturally-adapted and well-controlled system at national and international level. Public education and information about the ethical issues raised by stem cell research and its application is necessary.

O-60: Donation and Surrogacy from Islamic View

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Introduction: So far, there are too many children are born by donation protocols or surrogacy. In Islamic countries upon the third conference of the Islamic Fiqh Council in 1986, all kinds of donations are banned. So, no Islamic country practices donations except Iran in which, all of them are practiced everyday. As Iran represents an Islamic country and most of the Iranian people are Muslims, there can be some social, legal and psychological concerns for the children born by these protocols that should be considered, discussed and clarified for avoiding future complications. This study outlines some proofs and documents for helping the Islamic law-makers, clergy leaders and professionals to pursue a practical guideline in this regard and maybe an upgrade to the previous statement.

Materials and Methods: As we can not find the equivalent terms in the Islamic resource, we review the Islamic resources mostly the Holy Quran to find some proofs and we could find out the Islamic ideas.

Results: 1) These protocols are compared with adultery in many papers and statements but it has been shown that they are totally different. 2) It has been stated that these protocols bring confusion of lineage that does not. 3) Some relations like intimacy have been discussed that is OK in these families. 4) Two vision of cell donation and multiple marriages have been discussed and proved that cell donation vision is accepted and no fake marriage is OK in Islam. 5) We suggest two-mother theory that clarifies these children lineage so other rights linked to the lineage upon Islam. 6) We underline the anonymity of the donor to avoid complication that may come if the donor identity is known, that upon Islam donors will be true parents also, and some rights goes back to them. 7) We have suggestions about the inheritance of the children born by sperm and embryo donation which can not be solved by ordinary Islamic law.

Conclusion: It has been shown that Islam can accept donation and surrogacy by presenting proofs and documents from Islamic resources and clergy leaders can pursue a practical guideline in this regard.

O-61: Implication of Explanatory Model of Iranian Women Sexuality in Development of Sexual Education Program

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O-62: Sex Selection for Non-Medical Purposes

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Sex selection of the future offspring is usually regarded as acceptable when medically indicated, in order to avoid the transmission of sex linked severe genetic diseases. The principle of sex selection for personal or social convenience opposes those who support women's right to reproductive autonomy and those who condemn any gender discrimination. Sex selection can be performed before conception by sperm separation, before pregnancy by selection of in vitro fertilized embryos, in early pregnancy by abortion after foetal DNA analysis in maternal serum, chorionic villous sampling, amniocentesis and above all after mid trimester echography. Most procedures concern selective abortion of girls, specially in India, China and South East Asia where it is considered that, for economic and traditional reasons, 100 million girls are missing. Most scientific societies have condemned abortion sex selection. Many countries, including India, have enacted anti sex selection abortion laws but very few have efficiently enforced these laws. Should these practices be tolerated in the name of cultural identity and economic hardship, or banned in the name of gender equality, is ethically debatable.

O-63: Is there a Limit to the Number of Embryos to be Transferred/Created for ART?

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Pregnancy rate after ART (Artificial Reproductive Technology) is abusively considered to increase in proportion of the number of embryos transferred. Indeed this is only true up to less than 5 embryos, and is highly dependant on the woman's age. Above all a policy of too many embryos transfer entails an excessively high number of multiple pregnancy, as high as 26-30%, including 3-4% triplets. The hazards of multiple pregnancy both for mothers and offspring led several countries to limit by law the number of embryos to be created/transferred during ART. A policy of a single embryo transfer, eventually followed, in case of implantation failure, by the transfer of a cryopreserved embryo during a next cycle, has proven to be significantly as efficient as the transfer of two fresh embryos, with a drastic reduction in multiple pregnancy. The advantage of the transfer of a single embryo at the blastocyst stage is discussed. FIGO Ethics Committee recommends an embryo policy transfer achieving a singleton pregnancy.

O-64: Payment, Contracts and Registration in Oocyte and Sperm Donation

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Today many children are born by ART and also a part of them by egg, sperm and embryo donation or surrogacy. There is a need for some guidelines to outline these children's rights and prevent future problem. These guidelines can not be totally universal because of cultural, religious and ethnical differences. As one of the most important part of donation and surrogacy is payment, which brings other problems and questions like:

human dignity, advertisement, contracts, registration, law and human rights.
Here we discussed the payment, contract and registration upon years of experience in kidney transplantation from living donor in Iran, which had faced similar problem and solved a large part of them.
Here are our results: 1) nobody can sell their body's part and it is against human dignity, so, all of these procedures should be called donation. 2) the payment should be considered as compensation of the donor for their time, drug usage, operation, lab tests… 3) as there is no business on human parts, there can not be a contract on donation, but a contract on compensation is OK. 4) governmental law can not make anyone to donate their body cells, but registration committee can prevent repeat of any contract cancellation by not accepting a donor who had cancelled a contract before. 5) a governmental registration center shall be established to register donors and recipients' data and save them for preventing future complication, we insist on anonymity of the donors and keep the privacy of the files. 6) registration center should be responsible for contract, payment and data protection, that our suggestion is to design computer network enabling each town to have a registration center. 7) there shall be a governmental law to prevent any advertisement or need notice on the walls of the centers or in newspapers and magazines.

O-65: An Ethical Framework for the Use of Humanoocyte for Stem Cell Research

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O-66: Reconciling Donor and Recipient in Oocyte Donation Programmes

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O-67: Health Seeking Behavior of Couples with Secondary Infertility

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Objective: To determine the factors affecting the health-seeking behavior of couples with secondary infertility in Karachi.
Design: A descriptive case series.
Place and Duration of Study: The data was collected from women attending infertility clinics in five tertiary care hospitals in Karachi from March to June 2003.
Patients and Methods: All currently married women, between the age of 15-35 years, with at least one previous conception, irrespective of outcome, attending an infertility clinic and consenting to participate in the study, were included. Women with corrective surgery on vagina and uterus, and cases of primary infertility, were excluded. Multiple logistic regression models were used to determine the association of various factors, affecting the health-seeking behavior, with statistical significance set at $p < 0.05$ for the covariates and the interaction terms between various factors.
Results: The women consulted multiple health care providers for treatment of secondary infertility. The main reasons for seeking treatment were couple's wish (54.2%), family pressure (22.6%) and want of a son by husbands or in-laws (20.4%). The most commonly sought providers were physicians (74.7%), Traditional Birth Attendants (TBA, 39.5%), Spiritual healers (26%), Hakeems (23%) and Homeopaths (17.2%). Most of the women who consulted non-physicians were illiterate (69.4%) as compared to those who consulted a physician (37.8%, p-value = 0.00). The non-physicians were more commonly consulted by women

belonging to low socioeconomic group. The posttreatment complications were more common among women who consulted non-physicians.
Conclusion: Pressure from husbands and in-laws compels women for consulting multiple providers. Health seeking behavior for infertility is affected by the literacy and socioeconomic status of the women.

O-68: Comparison between the Scientific and Koranic Explanation for Human Creation

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People have always been interested in knowing how they originated, and how they were born. Ancient people, filled with curiosity, developed many answers to these questions. All of what we know now about the development of the embryo within the uterus is based on the accumulated scientific knowledge achieved over the centuries and particularly due to advanced researches in resent years after the development of the modern technology. Scattered through out the Koran are many statements about embryology.
The progress of embryology through the ages from the earliest records to the present and Enlightenment in this field in the holy Koran and Hadith are discussed. Read in the name of your Lord who created. Created man from a clot. (Al alaq 2-1)
This is the first Aya in the Holy Koran and this is the first stage in human Creation. In the Holy Koran God gave, 14 centering ago a detailed description of human creation from clay, ending with death, then resurrection in many verses (Aya).
Conclusion: The holy Koran was the first to exactly describe human development 14 centuries ago, long before the discipline of Embryology was even established. Only in the last century, modern science was able to prove in detail and with evidence what was reveled, in TRUTH, in the Holy Koran.

O-69: Analysis of National Representative Opinion Surveys Concerning Gestational Surrogacy in Japan

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Objective: Although gestational surrogacy offers several advantages, this procedure has given rise to some ethical and legal issues.We aimed to clarify the factors affecting the attitude of the Japanese toward gestational surrogacy.
Design: Cross-sectional study.
Setting and participants: Nationwide opinion surveys concerning assisted reproductive technologies (ART) were carried out in 1999 and 2003. Participants included 2568 and 3647 people from the general public surveyed in 1999 and 2003, respectively (1564 people received only the questionnaire, and 2083 people received a questionnaire and brochure about ART).
Main outcome measure: Multivariate-adjusted odds ratio and 95% confidence interval from logistic regression models for factors affecting the attitude toward gestational surrogacy.
Results: In both surveys, approximately half of respondents approved of gestational surrogacy; 20–30% disapproved of the procedure. People with high socioeconomic status clearly expressed their opinion on this issue. A liberal attitude toward gender role promoted approval of gestational surrogacy; a liberal attitude toward family had the opposite effect.
Conclusion: Our findings suggest that socioeconomic status affects people's

expression of their opinion regarding this issue, while attitudes toward this procedure were influenced by individual belief. Considering socioeconomic status and diversity of individual belief is required for further discussion on this topic.

O-70: Life-Table Analysis of Artificial Insemination Pregnancy Rates for Couples with Male Factor and Idiopathic Infertility

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Background: In the summer of 2002, standard guidelines for the application of assisted reproductive technology were reported by a research group of the Ministry of Health, Labor and Welfare. The present study aimed to examine the relationship between the number of cycles of artificial insemination and the cumulative pregnancy rates according to the cause of infertility.
Materials and Methods: Patients who experienced their first cycle of artificial insemination during the period of January 1999- December 2002 were included in the study and were divided into a male factor infertility group and an idiopathic infertility group. Cumulative pregnancy rates resulting from artificial insemination with the husband's semen were calculated by the life-table approach.
Results: During the study period, 139 couples entered the assisted reproduction program and underwent 581 cycles. Significant differences were observed in cumulative pregnancy rates between the two groups.
Conclusion: It is recommended that couples with male factor infertility and who fail to conceive within six or seven cycles of intrauterine insemination, consider a modification of treatment strategy such as in vitro fertilization, because cumulative pregnancy rates of this group were reached at a plateau within six or seven cycles. In contrast, patients with idiopathic infertility, the cumulative pregnancy rates appeared to increase constantly with each subsequent cycle. It is important to consider modifications of treatment strategy in the light of the cause of infertility.

O-71: Gender Preferences and Demand for Sex Selection: A Survey Among Pregnant Women in Pakistan

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Background: In its recent report “Human Reproductive Technologies and the Law”, the House of Commons’ Select Committee on Science and Technology called for greater efforts to establish the potential demographic impact of sex selection across all sectors of UK society. Given the well-known preference for boys over girls among some communities, there is concern that a readily available service for social sex selection may upset the balance of the sexes. Of particular interest are the gen-der preferences and the demand for sex selection among Pakistanis.
Materials and Methods: We conducted a social survey on gender preferences and demand for preconception sex selection among 301 pregnant women in Karachi, Pakistan, using a self-report ques-tionnaire consisting of 13 questions.
Results: 41.5% wish to have a family with an equal number of boys and girls. 3.3% would like to have only boys, 1.0% only girls, 27.6% more boys than girls, 4.3% more girls than boys, and 22.3% stated that they do not care about the sex composition of their family. While 6.3% could imagine em-ploying cytometric sperm separation for social sex selection, 76.1% could not; 17.6% were undecided. 27.2% felt that social sex selection ought to be legal, 48.8% thought it ought to be illegal, and 23.9% were undecided.

Conclusion: Although Pakistani women do show a marked preference for boys over girls, the number of women will-ing to subject them selves to cytometric sperm separation appears to be too small to cause a severe imbalance of the sexes. However, further research among British citizens of Pakistani origin is needed to establish whether or not sex selection poses a serious threat to the sex ratio of UK communities.

Female Infertility

O-72: A Comparison of Follicular Response of Ovaries to Ovulation Induction after Laparoscopic Ovarian Cystectomy or Fenestration and Coagulation versus Normal Ovaries in Patients with Endometrioma

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Objective: To compare follicular response to controlled ovarian hyperstimulation (COH) between normal ovaries and ovaries previously treated by different laparoscopic techniques for ovarian endometrioma.
Design: A prospective randomized clinical trial.
Settings: University and private infertility clinic.
Patients: 81 infertile patients with either unilateral or bilateral endometrioma.
Interventions: For 65 patients with unilateral endometrioma, laparoscopic ovarian fenestration and coagulation was performed in 24 cases (group 1) and laparoscopic ovarian cystectomy in 41 others (group 2). In 16 patients who had bilateral endometrioma (group 3) cystectomy was done in one ovary and fenestration and coagulation in the contra-lateral side. All patients underwent COH with clomiphene-citrate and HMG in subsequent cycles.
Main Outcome Measures: Number of follicles in each ovary of above patients after COH.
Results: Mean number of follicles in group 1 was 2.6 ±1.6 in post fenestration and coagulation ovaries and 2.8±1.6 in normal ovaries. These figures were 3.2±1.1 in post-cystectomy ovaries and 3.2±1.7 in normal ovaries in group 2.In group 3 number of follicle was 2.9±1.1 in post-cystectomy ovaries and 3.05±1.3 after fenestration and coagulation. There was no statistical significant difference between these figures.
Conclusions: Response of ovaries to COH after laparoscopic ovarian cystectomy or fenestration and coagulation was the same. There was no difference in response to COH between normal ovaries and those operated by laparoscopic techniques mentioned above.

O-73: Microscopic Surgery of Fallopian Tube Using Laparoscope

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One of the main causes of infertility is tubal factor. Post surgical adhesions, sexual transmitted diseases, PID, and post partum infection, may lead to tubal and peritubal factor infertility. Surgical approaches to these problems were not successful and after introduction of IVF in the field of reproductive medicine, trend of most infertility specialists is toward ART. In early 1980s, the introduction of microsurgery by laparatomy has created new hopes for these patients. With advances in operative laparoscopy during the 90s surgeons shifted from laparatomy to laparoscopy for patients who needed an operation in abdomen and pelvis. However, microsurgery by laparoscopy is a difficult task and there are not many surgeons in the world that could perform this technique. Since 2003, we have started using laparoscopic microsurgery to treat tubal and peritubal factor infertility. Different surgical techniques such as end to end anastomosis, corneul anastomosis, salpingoovolysis, salpingoneostomy and fimbrioplasty, has been done by laparoscopic microsurgery. Here indications, surgical techniques, and success rates will be discussed and some videos of our operations will be demonstrated.

O-74: Pentoxifylline Therapy after Laparoscopic Surgery for Different Stages of Endometriosis, a Prospective, Double Blind, Radomized, Placebo-Controlled Study

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Objective: To evaluate the effects of pentoxifylline administration on the patients with different stages of endometriosis, for whom laparascopy was performed.
Design: prospective, double-blind, randomized, placebo-controlled clinical.
Design Classification: Canadian Task Force classification I.
Setting: University and private hospitals.
Patients: Eighty eight women, all with infertility, some with dysmenorrhea, dyspareunia, or pelvic pain, for whom laparascopic diagnosis of endometriosis was done and as the principal part of the treatment, appropriate surgery was carried out.
Interventions: The treatment group received 800 mg of pentoxifylline daily for six months immediately after surgery. The control group received placebo capsules. All of them were followed for one year thereafter.
Measurements and Main Results: A comparison of pregnancy rate and recurrence of signs and symptoms in the two mentioned groups was done. Forty three patients were studied in the pentoxifylline group, and forty five in the placebo one. The cumulative pregnancy rate was 39.5% and 35.6% in the treatment and control groups, respectively. The overall recurrence of signs and symptoms was 14% in the former group and 15.6% in the latter one. So there were no statistically significant differences between the two groups in the rates of pregnancy and recurrence (P values: .7 and .832, respectively). Neither there was any significant statistical difference between the same stages in the two groups regarding immunomodulation.
Conclusions: According to the results of this study, and while keeping in mind that appropriate and perfect operation is the main aspect of the endometriosis treatment, there is no evidence that immunomodulation with pentoxifylline aids fertility, or recurrence of signs and symptoms in women with different stages of endometriosis (i.e., minimal, mild, moderate, or severe).

O-75: Menopur® vs Gonal-F® in Iran: A Cost Effectiveness Analysis Based on MERiT Study

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Multifollicular development via Gonadotrophin administration is still an integral component for ovarian stimulation in IVF/ ICSI cycles1.
The technological developments of pharmaceutical Gonadotrophins over the last 40 years have shown improvements in specific activity, purity, degradation and impurities2 and recently with the introduction of Highly Purified Menotropins (HP- hMG), both effectiveness and cost effectiveness should be considered together to aid the judgment about whether one drug should be preferred to a comparator3.
To examine the cost effectiveness of rec-FSH versus HP-hMG in Iran, it was necessary to build a model that simulates the IVF treatment cycle with its key steps Because of the recurring nature of IVF/ ICSI cycles, it was decided to run a model called Markov analysis model for three successive cycles. The transition probabilities for different health states used in the present analysis were based on results from a recently published randomized controlled trial called MERiT®4,5, for estimation of the cost of live birth in IVF/ICSI cycles. In the case of using rec-FSH, the live birth rate is 31.8%, while when using MENOPUR®, the live birth is 45.2% with an incremental cost effectiveness ratio ICER of 12,506,340 IR ~ 1050 EURO per life birth in favor of MENOPUR®. Cost effectiveness analysis reveals that MENOPUR® is better leading to Live birth rate state than that of rec-FSH

O-76: Comparison of the Efficacy of the Aromatase Inhibitor Letrozole

and Clomiphene Citrate as Adjuvants to Recombinant Follicle-Stimulating Hormone in Controlled Ovarian Hyperstimulation: A Prospective, Randomized, Blinded Clinical Tria

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Objective: To study the efficacy of the aromatase inhibitor letrozole as adjuvant to recombinant FSH (rFSH) in controlled ovarian hyperstimulation (COH). DESIGN: Prospective, randomized, and blinded clinical study.
Setting: Academic tertiary institute.
Patients: Forty-one patients with unexplained infertility undergoing intrauterine insemination (IUI) therapy were randomized to receive either letrozole or clomiphene citrate (CC) as adjuvants to rFSH.
Interventions: From day 3 to 7 of the cycle 2.5 mg/d letrozole or 100 mg/d CC were administrated. All patients received 75 IU rFSH starting on day 7 of stimulation until the day of hCG administration. Ovulation was triggered with recombinant hCG (250 microg) when the leading follicle(s) reached 18 mm in diameter. A single IUI was performed 36 hours later. The luteal phase was supplemented with micronized progesterone vaginally.
Main Outcome Measures: Ovarian stimulation response (E(2) levels and number of follicles) was our primary outcome.
Results: There were no differences in demographic characteristics between groups. Although there was a significantly lower peak serum E(2) level in the group receiving letrozole + rFSH compared with CC + rFSH (914+/-187 vs. 1,207+/-309 pg/mL, respectively; p<.007), there were no differences in the number of mature (>16 mm) preovulatory follicles. A significantly higher endometrial thickness was observed at the time of hCG administration in patients that received letrozole (9.5 +/- 1.5 mm vs. 7.3 +/- 1.1 mm; p=0.0001). The clinical pregnancy rate was similar between groups (23.8% vs. 20%, respectively).
Conclusion: The aromatase inhibitor letrozole appears to constitute a good alternative to CC in patients with unexplained infertility undergoing gonadotropin-stimulated COH cycles combined with IUI therapy.

O-77: Fetal Consequences of Occult Maternal Conditions

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It is a medical truism that fetal health depends on maternal health. Several conditions such as adult diabetes and cardiovascular disease have been linked to fetal exposure to poor maternal health and maternal stress. These insights have sparked investigation into the fetal origins of adult disease (FOAD). When the maternal conditions that are recognized to confer fetal consequences are amenable to medical intervention, then consideration should be given to identifying and correcting these conditions prior to conception. This is especially important when conception requires medical assistance, fetal consequences are significant, when screening strategies exist that are readily available and reliable, or when the condition is infectious and preventable by vaccine. The task of ensuring fetal health is more difficult when the maternal conditions are occult.
The mechanisms by which fetal development is altered by maternal conditions are many and include epigenetic mechanisms that alter DNA methylation patterns and therefore have the potential to be multigenerational. A short list of occult maternal conditions for which screening might be of benefit includes maternal thrombophilias, impaired maternal glucose tolerance, stress, hypothyroidism, and nutritional deficiencies, especially those that cause folate deficiency. For instance, celiac disease may cause malabsorption, hyper- or hypothyroidism, and infertility due to oligo-ovulation. Newer diagnostic techniques allow us to screen for celiac disease noninvasively and it is important to consider

screening for celiac disease in women with infertility.

Another common but often occult maternal cause of compromised fetal development is hypothyroidism. Worldwide, goiter due to iodine deficiency is a common cause of hypothyroidism and cretinism. Other common causes of maternal hypothyroidism include autoimmune etiologies, including celiac disease, and stress. The mother is the sole source of thyroxine in early gestation and the predominant source during the entire gestation. Women with a known cause of thyroid deficiency due to organic conditions such as Grave's disease or Hashimoto's thyroiditis require an increase in thyroxine dose very early in gestation. Alexander EK et al showed that the mean thyroxine requirement increased about 50% by week 8 of gestation (New Engl J Med 2004; 351:241). Thyroxine requirements appear to be greater in women undergoing assisted reproduction and ovulation induction and in those with a multiple gestation. The primary consequence of inadequate fetal exposure to thyroxine is compromised neurodevelopment. There are many important genes that are regulated by thyroxine and this appears to be the mechanism by which inadequate thyroxine exposure compromises fetal neurodevelopment. Since psychosocial stress and inadequate nutrition compromise hypothalamic TRH drive and lead to subtle forms of functional hypothalamic hypothyroidism, it may be more important than previously recognized to mitigate poor maternal psychosocial and nutritional compromise. *In summary*, recent data on the importance of thyroxine for fetal neurodevelopment support the notion that there should be a high index of suspicion and a low threshold for screening for thyroid disease. Optimally, screening should occur before conception and should include TSH, free thyroxine, and possibly anti-thyroid antibodies such as TSI (thyroid stimulating immunoglobulin) and TPO (thyroid peroxidase). It is more difficult to screen for functional forms of hypothyroidism that accompany psychosocial and metabolic stress and thus amelioration of psychosocial and nutritional stress should receive high priority.

O-78: Stress-Induced Anovulation

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Functional hypothalamic amenorrhea (FHA) is characterized by a reversible reduction in GnRH drive and mild hypercortisolemia. The behavioral antecedents leading to the development of non-organic forms of chronic hypothalamic anovulation and amenorrhea are variable. Dieting and excessive exercise are often initiated to cope with subtle psychogenic challenges. It is accepted that what is deemed stressful is to a large extent idiosyncratic and individualistic. Further, women with FHA rarely report a single isolated stressor and FHA can develop in the absence of significant metabolic imbalance. We wondered, therefore, if metabolic and psychogenic stressors would be more deleterious to reproductive function when combined. We developed a monkey model to test the hypothesis that a combination of metabolic and psychosocial stressors would synergistically disrupt reproductive function. We recognized that the distinction between psychogenic and metabolic stress was, to some extent, artificial, because the corresponding increase in cortisol secretion that accompanies activation of the limbic-hypothalamic-pituitary-adrenal axis elicits obligatory metabolic adaptations regardless of initiating cause. However, therapeutic recommendations are often based on the notion that metabolic stressors are easier to identify and ameliorate than psychogenic ones, so it seemed important to try to determine the impact of metabolic versus psychosocial stressors alone and then in combination.

To date, our monkey data suggest that mild metabolic imbalance heightens reactivity to subsequent psychogenic challenge. Other investigators have shown that psychogenic stress heightens the impact of metabolic (exercise) challenge. However, the neural concomitants that transduce either metabolic or psychogenic stress into altered neuroendocrine secretory patterns or heighten

reactivity to mildly stressful events remain poorly defined. Our work in female cynomolgus monkeys suggests that the serotonergic axis differs in stress-sensitive and stress-resistant monkeys. We found that cerebrospinal fluid levels of CRH were comparable in FHA and eumenorrheic women, but that, contrary to expectations, beta-endorphin levels were lower in women with established FHA. Interestingly, CSF levels of cortisol were higher in FHA. If the set point for inhibition of the HPA axis were comparable in FHA and eumenorrheic women, one would expect that CSF CRH levels would be lower in FHA than in eumenorrheic women. Thus, our data suggest that women with FHA display central resistance to the negative inhibitory feedback effects of cortisol. The physiological, cellular, or molecular basis of this stress sensitivity and limbic-hypothalamic-pituitary-adrenal feedback insensitivity in FHA has not been identified, but neurotransmitter systems such as serotonin and GABA remain as candidates. FHA is, in theory, reversible. Based on our understanding of the psychological and behavioral correlates of FHA, we developed a 16-session program of cognitive behavior therapy (CBT) for women with FHA. We did not instruct women to alter exercise or diet habits, but we did address attitudes that underlie stress sensitivity. Women with FHA were randomized to CBT or observation. Over 85% of women treated with CBT recovered either full or partial ovarian function as assessed by weekly estradiol and progesterone levels, whereas only 25% of those randomized to observation displayed reproductive recovery. Those who recovered did not gain weight. In aggregate, our data suggest that metabolic variables alone are rarely the primary cause of FHA and that psychological care has the potential to reverse FHA and thereby spare women with FHA the invasive, risky, and costly procedures of ovulation induction or assisted reproduction.

O-79: Polycystic Ovary Syndrome: Disease or Adaptation?

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The etiology of polycystic ovary syndrome (PCOS) remains uncertain. The three most commonly accepted causes are: a metabolic alteration that results in insulin resistance; an enzymatic defect in the steroidogenic pathway that increases androgen production; and a primary defect of the hypothalamic GnRH pulse generator that increases LH and decreases FSH. Some have argued that PCOS is invariably linked to insulin resistance, but this explanation does not fully account for the gonadotropin aberrations and resulting anovulation that are so characteristic of this syndrome. Part of the uncertainty regarding the etiology of PCOS relates to its variable presentation. For instance, thin women with PCOS are less likely to display insulin resistance and more likely to have excessive androgen levels. If ultrasound criteria are used to make the diagnosis of PCOS, even thin, eumenorrheic women who are ovulatory may be diagnosed as having PCOS. Our research objective has been to determine the etiology of the anovulation characteristic of PCOS so as to refine therapeutic approaches. In an effort to find a parsimonious explanation, we are seeking to determine the role of insulin resistance and hyperandrogenism in the genesis of the gonadotropin aberrations characteristic of PCOS. Indeed, available data suggest that both androgens and insulin increase GnRH drive and that increased GnRH drive secondarily reduces FSH to levels insufficient to fully support folliculogenesis to the point of ovulation. These data argue for more than one cause of PCOS. These insights form the basis for individualizing treatment approaches. For those not seeking immediate fertility, common treatments include anti-androgens, insulin action modifiers, metformin, and oral contraceptives alone or in combination. For those seeking fertility, the use of metformin alone or in combination has been advocated. A recent randomized trial suggested that clomiphene was superior to metformin for the treatment of infertility due to PCOS (Legro RS et al. New

Engl J Med 2007; 356: 551). Treatment efficacy varies widely and likely reflects etiologic nonhomogeneity. Recent advances in our understanding of the link between metabolism and aging suggest that PCOS may represent an adaptation to low fuel environments. If so, then it will be especially important to focus on nutritional counseling as a means of ameliorating symptom progression.

O-80: Modern Etiopathogenetic and Diagnostic Criteria for PCOS

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O-81: Management of Hirsutism

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Androgens constitute an important physiologic component for the female, representing the principal precursors of estrogens and directly participating to the secondary sexual characters determinism and to the sexual identity and behaviour definition.
Androgens play several and important metabolic effects, acting, moreover, in the regulation of a number of the female organism functions.
A regular and adequate ovarian and adrenal production of that hormonal fraction is essential for the activation and the modulation of important physiologic functions as:
1) Maintaining and regulation of the normal steroidogenic processes, acting as fundamental precursors for the global production of sexual steroids;
2) Induction of the process of follicular atresia following the selection of the dominant follicle;
3) Trophism of some tissues (especially of skin, bone and muscles);
4) Modulation of libido and sexual procectivity, in synergism with psycho-emotive and social-behavioural factors.
However, a condition of hyperandrogenism, due to excess of the steroid production or to increase of its biological activity, may determinate serious negative consequences which, often, impair not only the aesthetic aspect but also the psychic area and the interpersonal relationships. Not less important are the repercussions on the metabolic asset and, in successive phases of the life, on the cardiovascular risk.
That situation appears more and more important considering that up to 10% of adolescent women complain with a symptomatology related to an excessive androgen activity, even if the correct prevalence of the phenomenon could be largely affected by the diagnostic criteria utilized.
With the term of "*hyperandrogenism*" are identified all these clinical-pathologic conditions characterized by the presence of over-physiologic levels of androgenic steroids and/or the increased receptor sensitivity at the level of target tissues with consequent exaltation of the final biological effect .
On the other hand, with "*hyperandrogenic state*" are identified all these clinical-pathologic conditions, with multifactorial aetiology characterized by:
1) Increased body hair growth in normally smooth skin areas and/or with only little hair follicles (face, thorax, abdomen, gluteus, alba line) and increased growth speed of the hair structures;
2) Increased sebaceous production of the skin (in areas normally rich in sebaceous glands) and at the level of the scalp;
3) Alopecia;
4) Variable degrees of insulin resistance and/or hyperinsulinemia;
5) Reduction of the circulating levels of HDL cholesterol and modification of its rate with total cholesterol;
6) Alteration of the normal processes of ovarian follicle genesis with increasing of follicular atresia and failure on the selection and dominance of pre-ovular follicle; increase of ovarian stroma, albuginea thickening and formation of follicular micro-cysts.
Excluding *neoplastic* and *hyatrogenic* aetiology the so called *functional* causes of

hyperandrogenism are usually characterized by a multifactorial aetiopathogenesis.
Policystic ovarian syndrome (PCOS) represents the most frequent cause of hyperandrogenism in post-pubertal age. This complex disendocriny, which presents chronicity and self-maintaing aspects, is characterized by a severe disarray of the principal intra- and extra-ovarian feed-back mechanisms, presenting with a wide clinical, morphological and endocrine-metabolic variability.
Among all the causes of hyperandrogenism, PCOS is the most important under the etiophatogenic and prognostic aspect, often associating with characteristic dismetabolisms as dislipidemia (low levels of HDL cholesterol, alteration of total cholesterol/HDL cholesterol rate), impaired carbohydrates tolerance, insulin-resistance, overweight and obesity.
According to that condition several therapeutic options have been proposed over the years.
General measures are represented by eliminate causative factors, optimizing weight and manage hair with bleaching, cutting or shaving, electrolysis, laser epilation, but nowadays the most utilized and efficacious are represented by the etiphatogenetic therapy:

1) Ovarian andorgens production inhibitors (oral contraceptives, GnRH analogues);
2) Androgens receptor antagonists (ciproterone acetate, flutamide);
3) 5-alpha-reductase inhibitors (finasteride, dutasteride);
4) Insulin sensitizers (metformin, acarbose).

Oral contraceptives can be administered alone or in association with antiandrogens, insulin sensitizers or GnRH analogues.
Ciproterone acetate has been largely utilized with great success. It can be administered in low dosage being effective on acne/seborrhoea more than in hirsutism. The inverted sequential regimen according to Hammerstein, in association with low dose of estrogens, seems to be effective with Ferriman & Gallway hirsutism scores higher than 15, in presence of androgenic alopecia or impaired tolerance to estradiol (>20mcg). Low dosage of ciproterone acetate in association with GnRH analogues are often utilized in the treatment of poor responsive women in assisted reproduction protocols. Finally, low dosage in association with estrogens show same effectiveness vs GnRH-A characterizing as secondary choice in severe cases, for the absence of pituitary suppression.
Long term administration of ciproterone acetate plus contraceptive pill may, however, determine side effects as headache, nausea, depression, weight increase, hepatic function impairment and increase in triglycerides blood level.
More innovative appears the approach with insulin sensitizers. Several research demonstrated the key pathogenetic role played by hyperinsulinemia in the ovarian androgen overproduction of PCOS. On that purpose the reduction of hyperinsulinemia ameliorate the androgen excess.

O-82: Recombinant FSH Filled by Mass: A Scientific Step for Clinical Improvement

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O-83: Optimization of Ovarian Stimulation for Chronic Anovulation

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O-84: Entering New Era in Reproductive Medicine

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O-85: GnRH Antagonist: The New Standard for COS Protocols

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O-86: Endometrial Inflammatory Factors and Embryonic Implantation

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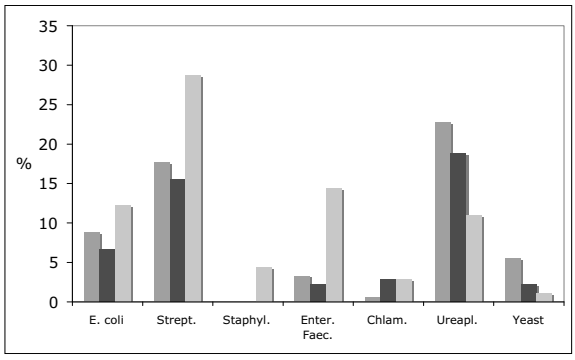
Chronic endometritis (CE) represents a chronic inflammation of the endometrial lining. In most of cases diagnosis is casual on histological specimen taken for different gynecological indications (AUB, infertility etc.). CE in most of cases is asymptomatic or accompanied by mild disturbances like spotting or bleeding (94% of cases), mild and undefined pelvic pain, mild leucorrhoea. Its prevalencerange from 0.5 to 15% in general population, while is 70% in women referring to centres for sexual transmitted diseases, often representing an hidden pathology.
The gold standard for the clinical definition and diagnosis of CE is represented by histology. The diagnosis of chronic endometritis relies on identification of plasma cells in the endometrial stroma. Lymphocytes, neutrophils, histiocytes, and eosinophils, are generally not diagnostic for endometritis, because they are normal constituents of the stroma. Inflammatory cells may infiltrate the wall of glands and may concentrate around vessels. The inconvenient factor is that these aspects may normally be present at menstruation and so diagnosis at histology may be missed.
As regards the pathophysiology, an abnormal uterine bleeding (AUB) can be observed from 70 to 90% of cases in which histology results positive for CE (Greenwood, 1995), accompanied by symptoms such as chronic pelvic pain, infertility and, less frequently, leucorrhoea and/or fever.
In a recent Italian study it was reported that the indication at hysteroscopy in 438women diagnosed with CE and 100 controls was 42% and 22% for dysfunctional uterine bleeding, respectively for CE and controls; 10% and 38% for positive transvaginale ultrasonography for endometrial polyp; 36% and 8% for infertility; 6% and 23% for submucous myoma and only 4% and 19% for mullerian abnormalities. These results show the significative relation between CE and AUB, and between CE and infertility.
According to the pathophysiology of the endometrial bleeding, in accord with the studies by Ferenczy, during a normal menstruation, in which the upper 2/3 of the endometrial mucosa detach, the endometrial cells and inflammatory cells produce proteolytic lysosomial enzymes causing a tissue breakdown whit consequent tissue necrosis, disruption of microvasculature, migration of leukocytes and platelet/fibrin thrombosis in microvessels.
On the other hand, during AUB, only the superficial layer (subsurface) detaches, diffusely (withdrawal bleeding) or focally (breakthrough bleeding). In the presence of the second evenience, CE and/or microerosions or vascular fragility due to structural abnormalities of microvessels could be frequent. However endometritis and microerosions may occur in otherwise normal endometrium, polyps, submucosal leiomyomata, atrophy and cancer (organic causes).
In that condition, Gram negative bacteria, mycoplasma and other infective agents may evoke a Th-1 response with endotoxin, macrophages, IL-1, and TNF-alfa local secretion, determining a pro-inflammatory response, responsible of damage of the conceptus, implantation failure, spontaneous abortion and preterm delivery, when acting at endometrial level, and hypercontractility and pain when acting at myometrial level.
The clinical diagnosis of CE is quite difficult. Physical signs are represented by uterine tenderness, spontaneous and at mobilization, leucorrhoea, cervical cyanosis, easy intracervical bleeding, but in most of cases is totally asymptomatic. Ultrasounds not specific signs are usually represented by endometrium out of phase, increased endometrial thickness and presence of endometrial fluid.
In consideration of that, the role of hysteroscopy appears not only important but necessary. At CO_2 hysteroscopy CE is

characterised by areas of red endometrium, flushed, with a white central point, localized or scattered out the cavity with the typical "strawberry aspect"described by Cravello.
More recently for that purpose, Cicinelli proposed the fluid diagnostic mini-hysteroscopy which characterizes for being painless and safe, performable also in case of bleeding (continuous-flow), easy to perform (learning curve shorter than with gas hysteroscopy), smooter distention of the uterine cavity (less pain), low costs and floating of ingrowths, with possible detection of subtle lesions. In detail, while saline solution allows floating of ingrowths while CO_2 causes flattening of ingrowths against the endometrial surface and, therefore, the diagnostic images obtained at fluid hysteroscopy may do not correspond to those described at traditional CO2 hysteroscopy.
Cervical signs of CE in course of fluid mini-Hysteroscopy may be represented by hyperemia, easy bleeding, cervical polyps, micropolyps, adhesions and leucorrhoea. On the other hand, endometrial signs are endometrial edema in the follicular phase (out of phase endometrium), diffuse or focal hyperemia (periglandular), endometrial polyps, adhesions and micropolyps, often representing, however, subtle lesions of difficult identification.
Endometrial micropolyps are described as an atypical aspect of endometrial surface characterized by the presence of very small (less that 1 mm of size) peduncolatous polyps showing a vascular axis. These subtle lesions may be sporadic or may cover most of the endometrial surface.
According to Cicinelli, 93.7% cases of micropolyps showed CE at histology and endometrial micropolyps are detected in 53.6% of all cases of CE at histology.
The presence of micropolyps shows a sensitivity of 54% and a specificity proxime to 99%, and the likelihood to have histological diagnosis of CE for women with micropolyps is very high (O.R. 124.2, C.I. 50.3 to 205.4).
As regards the etiology, the prevalence as percentage of infectious agents detected at vaginal (blue bars), at endocervical (red bars) and at endometrial cultures (yellow bars) in women with signs of chronic endometritis at hysteroscopy, according to Cicinelli et al. is represented in the following figure.



These studies have been prevalently oriented at the definition of CE in order to investigate its role in the blastocyst implantation process.
The human reproduction represents, in fact, a classical paradox, according to which it is critical to the survival of the species but relatively inefficient.
Maximal fecundity (the probability of conception during one menstrual cycle) is approximately only 30% (Zinaman et al., Fertil Steril 1996); only 50 to 60% of all conceptions advance beyond 20 weeks of gestation (Wilcox et al., N Engl J Med 1988); of the pregnancies that are lost, 75% represent a failure of implantation and are therefore not clinically recognized as pregnancies (Wilcox et al., N Engl J Med 1988); failed implantation is also a major limiting factor in assisted reproduction (Spandorfer & Rosenwaks, 1999); delivery rate per retrieval is 29.4%.
The implantation process occurs 6-7 days after conception (fertilization) and, in primates, includes 3 stages:
Apposition: initial adhesion of the blastocyst to the uterine wall. Microvilli on the apical surface of syncytiotrophoblasts interdigitate with microprotrusions from the apical surface of the uterine epithelium (pinopodes).
Adhesion: characterized by increased physical interaction between the blastocyst and the uterine epithelium.
Invasion: syncytiotrophoblasts penetrate the uterine epithelium. By then, the blastocyst is oriented with its embryonic pole toward the uterine epithelium.
The uterine receptivity represents the state during the period of endometrial maturation when the blastocyst can become implanted and the optimal period for implantation ranges between days 20 to 24 of a regular 28-day menstrual cycle. Uterine receptivity features include: endometrial histologic changes, more vascular and edematous glands, enhanced secretory activity; pinopodes develop on the

luminal surface of the epithelium and myometrial activity changes.
Successful implantation is the end result of complex molecular interactions between the hormonally primed uterus and a mature blastocyst and the failure to synchronize the component processes involved in these interactions may results in a failure of implantation.
Implantation factors, apart from the hormonal influence by estradiol and progesterone, include peptide hormones, growth factors and cytokines acting with autocrine/paracrine activity, involved in the cross-talk between endometrium and blastocyst.
According to these new acquisitions, leukemia inhibiting factor (LIF) could be considered as a new markers of endometrial receptivity. In detail its levels in both the luminal and glandular epithelium of the uterus rise dramatically in the midsecretory phase of the menstrual cycle and a diminished secretion of this factor is associated with recurrent pregnancy loss. Similarly, also adhesion molecules (integrins) and mucins are being considered valuable markers of endometrial receptivity.
Mechanisms that enable the blastocyst to actively initiate implantation include: catecholestrogens, leukemia inhibiting factor, transforming growth factor, platelet-derived growth factor, insulin-like growth factor II, colony-stimulating factor 1, interleukin-1, interleukin-6, prostaglandin E2, platelet-activating factor and epidermal-growth-factor receptors and heparan sulfate proteoglycans able to interact with factor–like ligands.
The finest understanding of the processes involved in inflammatory factors could be helpful to better manage the embryonic implantation failures in assisted reproduction and could pave the way to innovative diagnostic and therapeutic protocols which, in the next years, could be responsible of a pregnancy rate increase, in opposition to the "human reproduction paradox" quoted before.

O-87: 3D and 4D Sonography in the Assessment of Infertile Women

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O-88: Embryology and 3d Sonoembryology

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O-89: Expression of Melatoninergic Receptors in Human Placenta and Choriocarcinoma Cells

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Background: Melatonin concentrations increase in maternal blood during pregnancy, reaching a maximum at term. Moreover, experimental data suggest a possible influence of melatonin on placental function and fetal development in humans. To date, the expression and role of melatonin receptors in human placenta choriocarcinoma cell lines and in human term placental tissues remain to be elucidated. METHODS AND **Materials and Result:** Results from RT–PCR, western blotting and confocal microscopy demonstrated that the MT1, MT2 and RORa1 melatonin receptors are expressed in the human term placental tissues and in choriocarcinoma cell lines JEG-3 and BeWo (both *in vitro* models of human trophoblast). Furthermore, enzyme-linked immunosorbent assay showed that 6-chloromelatonin (a melatonin agonist) inhibits, in a dose-dependent manner, forskolin-stimulated hCG-β secretion in JEG-3 ($p<0.001$) and BeWo ($p<0.05$) cells but had no effect on basal human chorionic gonadotrophin (hCG-b) levels. This effect of 6-chloromelatonin on forskolin-stimulated HCG-β secretion was abolished by pertussis toxin (PTX),

suggesting that melatonin regulates hCG̃β production by an action involving an inhibitory Gi/o protein. In PTX-treated BeWo cells, 6-chloromelatonin stimulated basal hCG-β secretion ($p<0.001$).

Conclusion: These results demonstrate, for the first time, the expression of melatonin receptors in human term placental tissues and in choriocarcinoma cells and suggest a possible paracrine/ autocrine/ intracrine function for melatonin in human placenta.

O-90: Dendritic Cells and Pregnancy: A Bidirectional Relationship to Protect the Semiallogenic Fetus

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Dendritic cells (DCs) constitute a system of antigen presenting cells (APC) that are key regulators of immune responses. DCs are not only critical for induction of primary immune responses, but depending on their subsets and their maturation state may also be important for the induction of immunological tolerance, as well as for the regulation of T cell – mediated cytokine profiles.

Regarding the importance of Th2 immunity as well as induction of tolerance to fetal allograft during pregnancy and having considered the mutual role of DCs in induction of immunity vs. tolerance and Th1 vs. Th2 immune responses, it seems that these cells could be potentially suitable candidate that mediate the balance of maternal immune responses to support pregnancy. To address this issue we set out a series of studies concerning the role of DCs in indispensable immuneregulation during successful pregnancy and the effects of pregnancy related soluble factors (hormones, cytokines etc.) on DCs.

First of all we evaluated the frequency, localization and immunophenotype of murine endometrial DCs during different stages of estrous cycle. In addition, to address the systemic effect of hormonal fluctuations during estrous cycle, the same variables were studied in splenic DCs. The second study was performed to evaluate the kinetics of endometrial DC subsets at different stages of murine pregnancy. To address the systemic effect of pregnancy on DC kinetic, the same variables were also studied in splenic DC populations. The third study was set out to show the effects of soluble factors released in the supernatant of murine decidual cell cultures on the capacity of dendritic cells to present antigens in-vivo and on their ability to induce cytokine production by primed lymphocytes. We also investigated the immunosuppressive effect of pregnant mouse serum on allostimulatory activity of DCs to determine how activity of DCs could be affected during pregnancy. Regarding the pivotal role of Indoleamine 2, 3 dioxygenase (IDO) enzyme in pregnancy and considering that DCs are one of the main sources of this enzyme, the next studies were performed to investigate the effects of pregnant serum and decidual microenvironment on IDO induction in DCs and the role of IDO in DCs induced immunoregulation.

Various methods including: cell separation methods (mostly splenic DCs and lymph node T cells), cell and tissue culture methods, Mixed leukocyte reaction(MLR), lymphocyte transformation test(LTT), ELISA, cytochemical and immunohistochemical methods, morphometric analysis, flow cytometry, HPLC etc. were used to perform these studies.

Our data demonstrate that the balance of DCs subsets is finely tuned throughout estrous cycle and pregnancy. The frequency of endometrial DCs is highest at estrus, a phase in estrous cycle in which mating occurs and copulation is associated with further recruitment of large numbers of DCs in to the early deciduas, pointing an eminent immunoregulatory role of DCs in maintenance of pregnancy. It seems that soluble factors produced by decidual cells are important mediators of immunoregulation at the feto-maternal interface which provide the two fundamental requirements for protection of semiallogenic fetus, namely immunologic tolerance and predominance of Th2 immunity, through modulation of DCs function. Pretreatment of DCs with supernatant from decidual cultures but not pregnant serum significantly induces IDO expression while both treatments inhibit their capacity to induce production of TH1 cytokines and allogenic T cell allostimulatory capacity.

In conclusion, it can be postulated that DCs are key regulators of immune system during pregnancy and bidirectional relation of DCs and pregnancy protects the semiallogenic fetus from immunologic rejection. Such information may provide the basis for better understanding of female genital tract-immunity and may shed light on the way through which immunologically supporting microenvironment is organized for pregnancy maintenance.

O-91: Hysteroscopic Metroplasty of the Complete Uterine Septum, Duplicate Cervix, and Vaginal Septum

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Objective: To determine if sectioning of cervical septum in hysteroscopic metroplasty of the complete uterine septum is associated with intra-operative bleeding, cervical incompetence, and secondary infertility.
Design: Multicentric, randomized, controlled clinical trial.
Setting: University hospitals.
Patients: Twenty-eight women with the diagnosis of complete uterine septum who had history of pregnancy wastage or infertility. They were randomized into two groups; Group A; underwent metroplasty including section of the cervical septum.Group B: Underwent the same procedure with preservation of the cervical septum.
Interventions: Hysteroscopic metroplasty performed for all patients in the two groups.
Main outcome measures: Operating time, Distending media deficit, total distending media used, intra-operative bleeding, complications, and reproductive outcome.
Results: Operating times were 36.40±10.67 minutes and 73±14.40 minutes in group A and group B respectively (p<0.05). Distending media deficit was 456.66±165.68 ml in group A, while this variable for group B was 673.84±220.36 (p<0.05). Two cases of pulmonary edema and 3 cases of significant bleeding (>150ml) were seen in group B. Cesarean section rate was significantly higher in group B. There were no significant differences in the reproductive outcome in the two groups.
Conclusion: Resection of the cervical septum during hysteroscopic metroplasty of complete uterine septum makes the procedure safer, easier, and with less complication than the procedure with preservation of the cervical septum. This procedure is recommended for all cases of complete uterine septum.

P-92: Clinical Implications of hCG for Ovarian Stimulation

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The gonadotrophin preparations commonly used in assisted reproductive technology (ART) procedures contain similar follicle-stimulating hormone (FSH) concentrations but differ extensively in their luteinizing hormone (LH)-activity[1]. MENOPUR (highly purified human menotrophin) has the highest concentration of human chorionic gonadotrophin (hCG) – a source of long acting LH-activity – of all commercially available gonadotrophin preparations. The hCG-driven LH-activity found in MENOPUR has been demonstrated to positively influence ART outcomes at the level of the embryo and endometrium.
The European and Israeli Study Group (EISG) investigation and, more recently, the Menotrophin versus Recombinant FSH *in vitro* fertilization Trial (MERiT) established the impact of hCG-driven LH-activity in *in vitro* fertilization (IVF)[2,3]. In patients undergoing IVF, MENOPUR has shown equivalent safety profiles and a significantly higher live birth rate compared with recombinant FSH (rFSH)[4,5]. MERiT showed that the hCG-driven LH-activity in MENOPUR resulted in different hormone profiles compared with rFSH[6]. Progesterone levels at the end of stimulation

and on the day of oocyte retrieval were significantly lower in the MENOPUR patients compared with the rFSH group[6]. This has a probable impact on the quality of the endometrium and subsequently, embryo implantation. Additionally, it was shown that hCG levels on day 6 were highly predictive for ongoing pregnancy after IVF[7]. This suggests that hCG should be given from the beginning of stimulation, and possibly at higher doses, in patients who have reduced hCG levels on day 6 in order to increase the likelihood of pregnancy.

O-93: *In Vitro* Maturation of Oocytes

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In vitro maturation (IVM) of human oocytes is technically mastered for a decade. The patients groups included in the studies have been PCOS, male factor infertility and other causes of infertility. Both women with normal ovaries and PCO have been included. This method was developed in which pre-ovulatory immature oocytes were aspirated without gonadotrophin stimulation or low hormonal stimulation and matured in an incubator. To increase the pool of potentially competent oocytes and to make the oocyte pick-up easier, FSH priming has been used in some selected patients, primarily in women with PCOS. The results from randomized studies have shown conflicting results, some reporting better pregnancy rates with FSH priming and some showing no beneficial effect.
In vivo, gonadotropin plays an important role in the regulation of oocyte growth and maturation to trigger the meiosis resumption and nuclear maturation of oocytes in vivo. Recent studies reported that hCG priming before oocyte retrieval not only could enhance the oocyte maturation rate but also could improve the developmental potential of oocytes in vitro and increase clinical pregnancy rates. hCG priming also may aggravate nonsynchronization of oocyte nuclear and cytoplasmic maturation and which is considered to be a main reason of the poor development competence of IVM oocytes. So the effects of human chorionic gonadotropin (hCG) on oocyte maturation and development in vitro are still controversial. Of course use of hCG before immature oocyte retrieval in unstimulated ovaries make the maturation process faster and the number of MII oocytes will be higher, but there is no statistically demonstrable difference in the on-going pregnancy rate, endometrial parameters such as endometrial thickness, endometrial pattern ,or endometrial and uterine blood flow. In some centers hCG is generally added to culture medium when mammalian and human immature oocytes are cultured in vitro but recent studies indicated that the addition of hCG to in vitro culture medium did not improve the maturation rate or development potential of immature oocytes. For the IVM and development of immature oocytes from women with PCOS, hCG appears to be unnecessary.
In fact in IVM 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 hyperstimulation (COH). It can be expected that up to a certain extent, the in-vitro environment could be improved in a way to better support the final maturation stages of the oocyte. Nevertheless, the stages of oocyte development before oocyte-cumulus retrieval are equally important: it is during the earliest stages of follicle development that RNA and protein stores are foreseen for later development of the oocytes until implantation. Therefore the destiny of part of the oocytes is already determined at their retrieval. It is hypothesized that cumulus cells could reflect oocyte health and bear the markers for developmental competence. Rapid measurements for these markers could be of help to the embryologist to select the developmentally competent oocytes to include in culture.
Recent investigations have focused on the structural characteristics of chromatin and meiotic spindle of IVM oocytes, but little research was done concerning their chromosomal abnormalities, so their safe clinical use is still questionable. Recent

studies show the aneuploidy rate of IVM GV-oocytes is comparable to the aneuploidy rate of in vivo matured oocytes and first polar bodies, regardless of the length of maturation period.
Among young women affected by malignancy, the window of opportunity for fertility preservation is often limited by inadequate time to undergo ovarian stimulation or contraindication to hormonal stimulation in certain type of cancer. For these patients immature oocyte retrieval and IVM were performed prior to commencing cancer treatment. This is a new indication of IVM and embryo cryopreservation.
Therefore 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.

O-94: A Study of Ovarian Autitransplantation without Vascular a Pedicle in Rats

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Objective: The study aimed to determine effects of FSH applications on follicle survival, development and hormone output and antigenicity of rat ovarian tissue autografts placed at subcutaneous or subperitoneal sites.
Materials and Methods: A total of sixteen female rats were used in the study. The animals were divided into three groups. Ovaries were dissected and then transplanted under the peritoneum in the first group animals (n = 5) or under the skin in the second group animals (n = 6). And the animals in the third group (n = 5) were sham operated. Following operations, intramuscular injection of 8 IU of rhFSH were made daily to the animals in first and second groups from the first day of operation through thirty days. Vaginal irrigation samples were prepared daily from the animals for 30 days. The concentrations of serum estradiol and antiovarian antibodies in the blood were determined using ELISA on the last day of vaginal irrigations. Histopathological examination of the ovaries that were transplanted was made.
Results: Results showed that cyclic variations were noticed in the samples of vaginal irrigation by day 30 in the animals of first and second groups. However, no significant differences were seen between groups. The concentration of blood serum estradiol was higher in the animals of first group. Decrease in numbers of primary follicles were found in the animals of second group and lesser corpus luteum were found in the animals of control group on the histopathological examinations of transplanted ovaries. All rats in the first and second groups were defined as seropositive for antiovarian antibodies. When the OD values were compared between first and second groups, it was identified that the OD values of rats in the first group was higher than it was seen in the second group.
Conclusion: The ovarian transplantation without vascular pedicle in rats is characterized by follicular hyperplasia endocrinologically functional. Being seropositive of all rats in first and second groups in terms of antiovarian antibodies is an indicator to these antibodies does not affect the functions of transplanted ovaries. It is believed that the highness of OD values in the group which is transplanted beneath the peritoneum is based on the highness of estradiol concentrations in these animals.

O-95: A New Proposed Technique for Chorionic Villus Samling (CVS)

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Introduction: One of the techniques used for prenatal diagnosis is CVS. In contrast to amniocentesis (AC) carried out between 16 to

18 weeks gestation (second trimester), CVS can be done before 12 weeks of gestation (first trimester). Currently CVS can be done using both trans-abdominal (TA) and Transcervical (TC) techniques. Both methods are done between 9th and 11th weeks gestation under the ultrasound guidance. There is however controversy as to which technique is better, although TC is preferred when placenta is in a posterior position. Compared to amniocentesis, there is a higher miscarriage rate following CVS. Brambati et al (1991), in a randomized study showed that miscarriage rate in CVS done by TA was 16.5% compared to 15.5% in TC method. Various studies have demonstrated that CVS done by TC encounters more bleeding and sampling might need repeating. Since the current CVS methods inherit some problems such as bleeding and miscarriage, there is therefore a need for a safer method. In this study a new technique for CVS using transvaginal myometrium (TVM) has been introduced.

Materials and Methods: we investigated 362 patients referred to Sarem hospital from different genetic centers in Tehran. CVS was carried out on the patients using either TC or TVM randomly. All the patients were monitored by ultrasound and those with demised fetus, unsuitable anatomy, occurrence of bleeding in the previous week, twin pregnancies, and cervical fibroma were excluded from the study. The patients were aware of the CVS technique and its disadvantages. For TVM procedure the patients were placed in a lithotomic position. The vagina was cleaned using sterile swab and physiological saline. The position of fetus and placenta was monitored using ultrasound with vaginal probe. After positioning placenta in the movement path of a needle placed in a special guide and over the vaginal probe, the needle was directly inserted in the placenta through adjacent furuncles and myometrium. A 20 ml syringe containing 5cc culture medium and heparin was attached to the needle. Using negative pressure the villus was aspirated inside the syringe and with the same pressure, the needle was removed from the placenta and myometrium. Using dissection microscope the villus was checked and then the patient was transferred to the recovery room. CVS using TC was done using standard techniques.

Results: For three patients CVS was repeated on the same day. The average age of the patients was 28.9± 7.4. years. CVS was carried out at 10.8± 1.5 weeks. Following CVS with TC method, there was one miscarriage, one oligohydramnio, and one case with bleeding. In contrast TVM technique had no adverse effect.

Conclusions: CVS using TVM technique seems to be a safe technique and a better alternative to TC. However for a more conclusive result, further studies using a bigger sample size is warranted.

O-96: Differences in Endocrine and Paracrine Milieu Induced by HP-hMG (MENOPUR) Compared to rFSH (GONAL-F) Influencing Life Birth Rate

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It has been demonstrated in several models that the LH activity brings a dimension of quality in the cohort of follicles recruited by FSH. Evidence is accumulating that a supplementation of LH-activity independent of endogenous LH levels might be beneficial for pregnancy outcome. Recent data suggest differences in efficacy of treating women with follicle-stimulating hormone (FSH) alone and gonadotrophins containing LH-activity.

LH plays a dual role in folliculogenesis. It promotes follicular development and maturation through the production of androgen and growth factors in theca and granulosa cells. Additionally, LH appears to be involved in the negative selection of smaller follicles within the first few days of the stimulation–probably via a shift in the androgen/estrogen balance.

Unlike recombinant follicle-stimulating hormone (rFSH), highly purified human menopausal gonadotrophin (MENOPUR) contains both FSH and human chorionic gonadotrophin (hCG), a source of long-acting LH-activity.

The Menotrophin versus Recombinant FSH in *in vitro* fertilisation Trial (MERiT®) showed a trend towards a reduced number of oocytes at retrieval, which nonetheless, developed into a significantly higher number of top quality embryos per oocyte retrieved with MENOPUR versus rFSH. The hCG-driven LH-activity in MENOPUR happens to be the key differentiator driving the selection towards better quality follicles for *in vitro* fertilisation cycles.

The different gonadotrophins lead to an endocrine profile with a major impact on the endometrium. MERiT found that progesterone levels were significantly lower on the days of hCG and oocyte retrieval with MENOPUR compared with rFSH and these lower progesterone levels were associated with higher implantation and ongoing pregnancy rates.

Further analysis of cumulus-corona cells in patients treated with MENOPUR or GONAL-F showed a significant difference in gene expression on the day of oocyte retrieval in approximately 500 genes. Some of the genes changed by Menopur are known quality genes expressed in cumulus after stimulation. This data (unpublished from the authors' laboratory) might lead to a better understanding of cumulus-corona cell function in relation to oocyte quality and is an interesting subject of research.

Together, these data suggest that the hCG-driven LH-activity present in MENOPUR brings a significant change in the close environment of the oocyte and in the hormonal balance that might influence endometrial receptivity, compared with rFSH.

O-97: Challenges of Ovarian Follicle Culture

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Follicle culture systems are interesting models to learn about animal and human reproductive physiology. The choice of the culture technique is based upon: the size of the ovary (the species), the developmental stage of the follicle of interest, and the duration of folliculogenesis. The success of a culture system is judged by its ability to produce developmentally competent oocyte. Until today only the mouse follicle culture is a successful system. This follicle culture model will be described. Problems associated with the large size of the ovary (human and domestic species) can be solved by culturing thin slices of tissue. Unfortunately, adult ovarian tissue is not the ideal material to attempt to develop a human follicle culture system as their follicle density is low. The stromal tissue in the ovaries of larger mammals is fibrous and dense, and therefore does not facilitate the development of a successful system for isolation and culture of follicles. More research still needs to be accomplished on small follicles. The major requirements for normal growth of follicles at the earliest stages are still unknown and must be determined if developmentally competent oocyte are to be finally obtained. Fetal ovaries are useful experimental material because of their high follicle density and the softness of the tissue. Healthy T layered follicles in culture grown from primordial fetal follicles were morphologically comparable to those grown in vivo in the vast majority of cases. However, cellular development under culture conditions might be more susceptible to genetic changes. Long-term culture could result in inappropriate cellular differentiation, genetic modification and formation of tumour cells.

O-98: Two Novel Techniques to Detect Follicles in Human Ovarian Cortical Tissue

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Background: Ovarian tissue cryopreservation and transplantation are becoming increasingly important issues for preserving female fertility as shown by recent successes in restoring ovarian activity and even fertility. Primordial follicle content before transplantation is a key issue for success. We investigated two novel methods to detect primordial follicles in human ovarian cortical tissue strips.
Materials and Methods: The first method used the fluorescent mitochondrial stain rhodamine 123 in combination with laser scanning confocal microscopy (LSCM). The second used a simple stereomicroscopic method with glass-bottomed dishes for detecting primordial follicles in cortical ovarian tissue strips. Potential toxic effects of R123 and of the exposure to confocal laser were investigated in a mouse ovarian allograft model.
Results: Follicles were visible as white spots in thin cortical strips using LSCM in single and fast scanning at low magnification, allowing a fair estimation of the number of primordial follicles present. Using the second method, ovarian follicles were also visible using glass-bottom dishes under the stereomicroscope, although tissue thickness and density were limiting factors of its success.
Discussion: Follicles can be visualized in human cortical ovarian strips with R123 in combination with LSCM. Stereomicroscopy using glass-bottomed dishes and transmitted illumination is a simple alternative method and has the advantage of allowing further safe clinical use of the analysed tissue.

O-99: Inflammatory Mediators of Endometriosis Pathogenesis

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Endometriosis affects about 70 million women world-wide and accounts for 1-2 billion US dollars in medical and surgical health expenses annually. A variety of theories has been promulgated regarding the etiology of endometriosis, yet the precise pathogenic mechanisms responsible for its associated pelvic pain and infertility remain unknown. The establishment of endometriosis lesions in extrauterine locations (eg, peritoneum, ovary or rectovaginal septum) requires the invasion and neovascularization of endometrial stromal and gland cells within the extracellular matrix of the ectopic foci. Recruitment of innate immune cells into endometriotic lesions and the peritoneal cavity is a key pathophysiological feature of the syndrome. Neutrophils and macrophages are attracted from the circulation via chemokines, including ENA-78, eotaxin, IL-8, MCP-1 and RANTES. This presentation will focus on the latter, a C-C or beta-chemokine with chemoattractant properties for monocytes, T cells and eosinophils that has been extensively characterized in our laboratory. Concentrations of RANTES are elevated in the peritoneal fluid of women with endometriosis and correlate with disease stage. Stromal cells in ectopic implants appear to be the primary cellular source of RANTES. Analyses of the human RANTES gene promoter in endometrial and endometriotic cells indicate that proinflammatory cytokines (eg, IL-1-beta and TNF-alpha) regulate RANTES expression via the activation of the transcription factor NF-kappaB. Estrogens and dioxins can increase RANTES production and appear to exacerbate endometriosis. Agents that inhibit NF-kappaB signaling reduce RANTES mRNA and protein production. Two general classes include certain non-steroidal anti-inflammatory drugs (NSAIDs) and thiazolidinediones (TZDs). These pharmaceuticals have been tested using *in vitro* cultures of human endometriosis cells and also in mouse and baboon models of endometriosis. These compounds show promise in the reduction of lesion volume. The findings of these preclinical studies promise that new opportunities for drug discovery exist and that refinement of treatment choices for endometriosis will emerge.

O-100: Endometrial Angiogenesis: Physiology and Pathology

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The *functionalis* layer of human endometrium is a highly dynamic tissue, undergoing dramatic cyclical remodeling throughout a woman's reproductive life. The uterine lining typically grows from a thickness of 0.5 mm in the postmenstrual phase to >5 mm in the late proliferative and secretory phases of the endometrial cycle. The robust proliferation of endometrial epithelial and stromal cells is accompanied *pari passu* by extensive vascular and capillary growth, which provides the oxygen and nutrients needed to support the cyclic tissue expansion. Excessive angiogenesis is associated with endometrial polyps and neoplasia, whereas reduced angiogenesis appears to lead to defects in endometrial receptivity, embryonic loss and pregnancy complications associated with failed placentation. Our laboratory has been interested in the endocrine factors that mediate capillary proliferation in the human uterus and we have focused predominantly of the role of vascular endothelial growth factor (VEGF). This gene product is the most potent mitogen for capillary endothelial cells and it is highly expressed in endometrial epithelial and stromal cells. We have determined that the human VEGF gene is under direct transcriptional control by estrogen receptor complexes liganded to 17-beta estradiol (E2) or synthetic estrogens. We identified a unique variant estrogen responsive element in the human VEGF gene promoter that mediates the stimulatory effects of estrogens on VEGF production in endometrial cells. Progesterone (P4) also imposes proangiogenic effects on endometrial cells via the upregulation of VEGF gene and protein expression, although its mechanism of action is more mysterious. Unbalanced progestins may contribute to abnormal uterine bleeding associated with contraceptives. The combination of E2 and P4, as observed in the midluteal window of implantation appears to optimize the vasculature for embryonic receptivity. By contrast, anti-angiogeneic therapeutics may be useful in conditions associated with excessive endometrial vascular growth, such as polyps, endometriosis or contraceptive break-through bleeding.

O-101: Human Embryonic Implantation: Mechanisms and Consequences

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Embryonic implantation in women, as in other eutherian species, is intrinsically inefficient. It is postulated that >30% of fertilized human embryos fail to implant in normal ovulatory cycles, and this ratio increases even further with embryos derived via assisted reproductive technologies. Human implantation requires precise coordination between blastocyst and endometrial development. In their classical studies of hysterectomy specimens collected in the 1950s, Hertig and Rock observed that prior to cycle day 20 (approximately 6 days post-ovulation [POD]), all identified human embryos were found floating in the reproductive tract. In IVF cycles, Bergh and Navot (1992) identified the window of implantation between POD 6-10, and in natural cycles, Wilcox et al. (1995) observed that pregnancy follows intercourse that occurs up to POD 6. Four key stages of human blastocyst implantation have been defined: apposition, adhesion, penetration and invasion. These are regulated, in part, by the degree of endometrial epithelial and stromal differentiation that dictate uterine receptivity. Disorders in these coordinated processes can result in a spectrum of clinical conditions ranging from failed fertilization and spontaneous abortion to preeclampsia and even to excessive placental invasion seen in gestational trophoblastic neoplasia. Our laboratory and others have been interested in endometrial proteins that serve as functional mediators and biomarkers of the implantation process. A variety of integrins, homeobox proteins and immune regulatory molecules have

been identified and these will be presented. The lecture will focus on the role of glycodelin A, an immunosuppressive glycoprotein, as a prototypical biomarker of the late stages of implantation. The structure, regulation and function of this protein will be reviewed and its clinical utility and potential therapeutic activities will be discussed.

O-102: Clinical Study of Different form of Hysteroscopic Surgery for Endometrial Polyps

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Objective: To investigate the curative effects of different types of hysteroscopic surgery for endometrial polyps.
Materials and Methods: A total of 327 cases by different ways by hysteroscopic surgery for endometrial polyps from Nov. 1999 to Nov. 2004 were followed up. The mean age was 40±6 years. The mean follow-up was 3±0.6 years. Among 228 polyps patients in sexual maturity without desire of fertility, 53 cases (Group A) underwent polypectomy plus electrosurgical vaporization, and 175 cases (Group B) did polypectomy plus endometrial resection. 54(Group C) cases (19 cases of infertility), who desired future childbearing, did polypectomy plus endometrial resection of superficial layer near the polyps. 45 postmenopausal patients (Group D) did polypectomy plus endometrial coagulation.
Results: The time of operation: Group A 15.14±0.76 , Group B 19.68±0.66 , Group C 20.89±0.72 , Group D 22.12±0.81. None of polyps recurred for the patients of Group A and D after operation, and the recurrent rate of Group B and Group C was 1.71% and 7.41%.As been asked to keep the function of pregnancy, there were no cases with amenorrhea in Group C, but the recurrent rate of polyps was higher than other three groups. Of 19 cases of infertility, 14 cases got pregnancy after the surgery.
Conclusion: it is feasible to select different hysteroscopic surgery for endometrial polypsaccording to different ages and the desire of childbearing.

O-103: Ovarian Interstitial YAG-Laser: An Efftive New Method to Manage Anovulation in Women with Polycystic Ovary Syndrome

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Introduction: To assess the effectiveness of transvaginal ultrasound-guided ovarian interstitial laser-coagulation treatment in anovulatory women with polycystic ovary syndrome (PCOS).
Materials and Methods: Twenty-three anovulatory women with clomifene citrate-resistant PCOS. underwent the ultrasound-guided transvaginal ovarian interstitial YAG-laser treatment. A fibre-optic cable of 400 um in diameter was sent into the predetermined intraovarian point by long needle. The electrical laser was projected persistently for 1-2 minutes with a power of 3-5W until 3-5 points were completed on each ovary. Serum LH, FSH, testosterone(T), Prolactin(PRL), and Estradiol(E_2) levels, spontaneous ovulation rate and pregnancy rate were measured over six months follow up.
Results: Regular ovulation occurred in 19 out of 22 (86.4%) in the six months following ovarian treatment (1 case failure to follow). On the postoperative 2nd, 4th and 6th month , the mean serum LH were 4.54±1.21IU/L, 4.90±2.18IU/L and 4.42±1.03IU/L, and significantly ($p<0.001$, $p<0.001$, $p<0.001$) lower than preoperative level of 13.89±3.62IU/L; the mean serum testosterone levels were 2.69±1.83nmol/L, 2.42±1.11 nmol/L and 2.28±1.96nmol/L, and significantly

(p<0.001, p<0.001, p<0.001) lower than the preoperative baseline value of 5.37±3.09nmol/L; the mean LH/FSH ratios of 0.93±0.26, 0.88±0.17 and 0.81±0.14, were also significantly lower than the preoperative value of 2.78±1.21 (p<0.001). Pregnancy occurred in 8 women and there was a cumulative pregnancy rate at 6 months of 36% (8/22) among the subjects. There were no significant operative complications.
Conclusion: Ultrasound-guided transvaginal ovarian interstitial laser treatment appears effective in improving hormonal profiles and inducing ovulation and successful pregnancy in women with clomifene-resistant PCOS.

Genetics

O-104: Assisted Reproductive Technology and Pre-implantation Genetic diagnosis for Genetic Disease-Free Children

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Assisted reproductive technology (ART) has achieved a unique position in modern health care services as a model application of basic biomedical science and technology to disease management that gave hope to millions of subfertile men and women allover the world.
The main clinical indication of ART remained for more than a decade restricted to the treatment of infertility. A breakthrough, however, was realized in the power of ART when merged with the revolutionary progress in medical genetics and genetic technology to extend a hand to another group of people who are fertile but disparate to have a healthy child free from genetic disease traits carried by a couple.
Pre-implantation Genetic diagnosis (PGD) in combination with ART has now helped thousands of couples for near 15 years with such problems.
As a leading medical center for ART technology, we realized early enough the potential and the need for PGD in region that has high prevalence rates of genetic diseases with some specific diseases like thalassemia and other hemoglobinopathies at the top list.
The social and economical burden on our society and the human suffering associated with such diseases prompted us to introduce the PGD technology to our center in 1999 first for chromosomal evaluation. This was followed later by establishing PGD for single gene defects exploiting different approaches of the powerful PCR technology. In addition to the relatively large number of PGD cases for chromosomal assessment performed at our center, near twenty cases of PGD for different hemoglobinopaties have been performed at our center with an acceptable percentage of successful perganancies and healthy born babies (by international standards) achieved.
We will review the history and clinical aspects of our PGD program in this presentation.

O-105: Chromosome Polymorphism in Infertile and Sub-fertile Patients: A Pilot Study

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Introduction: Genetic causes account for 10-15% of severe male infertility, including chromosomal aberrations and single gene mutations. Identification of genetic factors has become a good practice for appropriate management of the infertile couples. Therefore it is important to identify genetic markers which could affect fertility as well as assisted reproductive techniques. Chromosome polymorphism is usually considered as normal population variant with no clinical significance, although there are controversial reports regarding infertile and sub-fertile patients. The variants comprise heterochromatin regions with no coding potential, nucleolar organizing regions (NOR) on acrocentric chromosomes which contain genes coding for r-RNA and satellite regions

on acrocentric chromosomes containing repeated DNA sequences.
The aim of this study was to evaluate the correlation between chromosome variants and infertility and sub-fertility.
Materials and Methods: Cytogenetic analysis was undertaken in 63 patients referred for infertility and recurrent abortions. 100 patients referred for mental retardation were used as controls. High resolution GTG banding was carried out on all the patients. C-banding was performed to confirm heterochromatin polymorphism.
Results: The incidence of chromosome variants was 30% in infertile and sub-fertile and 35% in control patients (MR). The variants included increased heterochromatin size on chromosomes 1, 9, 16, and Y; large NOR and satellites in acrocentric chromosomes and pericentric inversion of chromosome 9.
Conclusion: It seems that the rate of chromosome polymorphism in infertile and sub-fertile patients is not different from patients with mental retardation. However, larger data for more conclusive results is warranted.

O-106: Embryo Methabolism and Culture Media: Impact on Apoptosis and Imprinting

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O-107: DNA Decays and their Repair in the Oocyte

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O-108: Poly (ADP-ribose) Polymerase-2 Contributes to the Fidelity of Male Meiosis I and Spermiogenesis

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Poly (ADP-ribosyl) ation is an immediate but transient DNA-damage-dependent post-translational modification of histones and other nuclear proteins that contribute to the survival of injured cells.
Beside the established central role of poly (ADP-ribose) polymerases-1 (Parp-1) and 2 (Parp-2) in the maintenance of genomic integrity, accumulating evidence indicates that poly (ADP-ribosyl)ation may modulate epigenetic modifications under physiological conditions.
Our recent study provide the first *in vivo* evidence for the pleiotropic involvement of Parp-2 in both meiotic and post-meiotic processes. We show that Parp-2 deficient mice exhibit severely impaired spermatogenesis with a defect in prophase of meiosis I characterized by massive apoptosis at pachytene and metaphase I stages. While *Parp-2-/-* spermatocytes exhibit normal telomere dynamics and normal chromosome synapsis, they display defective meiotic sex chromosome inactivation associated with an upregulated X and Y-linked gene expression. Furthermore, a drastically reduced number of crossover-associated Mlh1 foci is associated with chromosome missegregation at metaphase I. Moreover, *Parp-2-/-* spermatids are severely compromised in differentiation

and exhibit a marked delay in nuclear elongation. Altogether, our findings indicate that in addition to its well known role in DNA repair, Parp-2 exerts essential functions during meiosis I and haploid gamete differentiation. In addition, this study raises the possibility that an impairment of Parp-2 could be a novel and so far unrecognized cause of infertility in humans.

O-109: Regeneration Potency in the Animal Kingdom

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Many lower organisms have the capacity to regenerate after amputation. The more primitive the organism is, the better it is in regenerating. Many lower species can produce a regenerating blastema from which the missing tissues will develop. The principle is that dedifferentiated cells allow regeneration and the formation of totipotent stem cells, which can differentiate in all cell types. They can proliferate in response to amputation but are also required for tissue maintenance in adult animals.
Mammals have lost most of this regeneration capacity. Regeneration can be classified into tissue regeneration and epimorphic regeneration. Liver regeneration in humans is classified as tissue regeneration. Besides this rapid type of repair some higher vertrebrates still have some form of regeneration. This can be defined as a process that leads to the complete replacement of the injured structures, culminating in a complete restoration of tissue function. There are only a few examples of epimorphic regeneration in mammals. The regeneration of digit tips is one of the exceptions. One explanation for the absence of epimorphic regeneration in mammals is the absence of cells from which regeneration could occur. The ability to regenerate might predispose the organism to cancer. The question is whether these cells are either absent in higher organisms or mutated in such a way that they have lost the ability to regenerate. Furthermore, in mammals it is vital to stop bleeding and lower the risk of infection after an injury has occurred.
In this lecture regeneration in the animal kingdom will be demonstrated using examples from sponges, hydra, planaria, newt, mouse and man.

O-110: Genetic Aspects of Infertility and its Treatment

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Genetic diagnosis in case of infertility is becoming more and more important. Many types of cytogenetic studies can be carried out on metaphase chromosomes or on interphase cells. In the former case, classical karyotypes are made after mitotic arrest of dividing cells followed by a banding method. This is sufficient for the study of all numerical and most structural constitutive abnormalities. Non-dividing interphase cells can only be studied by the application of molecular techniques, such as Fluorescent in situ hybridisation (FISH).
Other promising approaches comparative genomic hybridisation (CGH) and (micro-) array genomic hybridisation (Array CGH). Array CGH is a tool that can be used efficiently to identify unbalanced subtelomeric rearrangements, microdeletions, marker chromosomes and aneuploidy, including low level mosaicism.
Mutations in monogenic disorders can be studied after “classical” PCR amplification of the DNA from the blood or any other tissue available (even single cells) followed by the application of mutation screening and sequencing techniques.
With the introduction of IVF and other forms of assisted reproduction, it has become possible to treat genetic forms of infertility and to diagnose the resulting early embryos. Preimplantation genetic diagnosis is nowadays an established diagnostic procedure, which makes use of diagnostic methods, such as fluorescent in-situ

hybridization (FISH). Further technical developments leading to the
introduction of array-based technology might be expected soon to result in methods that allow the diagnosis of all aneuploidies at the same time in the same single cell. This will not only lead to better approaches for preimplantation genetic screening but
also help in a better understanding of how chromosomal abnormalities may explain the natural limits of human fecundity.
This has also clear implications for where the limits of success may lie for IVF.
Also the introduction of PCR has contributed tremendously to diagnosis at the single-cell level but also to answers regarding very fundamental questions in the field of the genetic programming of early development.
Finally the application of ART at the interface of reproductive medicine and medical genetics will be discussed in relation to the risks involved. Since sometimes important steps of natural conception are bypassed and immature gametes may be used, chromosomal and epigenetic defects might be more frequent in the offspring of ART conceptions than in the normal population. These genetic risks for the embryo will discussed.

O-111: Novel Mutations and (TG) m (T) n Polymorphism in Iranian Males with Congenital Bilateral Absence of the Vas Deferens

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Congenital bilateral absence of the vas deferens (CBAVD) is a frequent cause of obstructive azoospermia. Nearly 75% of men with CBAVD have at least one detectable common *CFTR* mutation. To study the *CFTR* gene mutations and (TG)m(T)n polymorphism in Iranian CBAVD patients with presumed low cystic fibrosis (CF) frequency, and to better understand the complex regulation of exon 9 splicing between our studied population, we analyzed *CFTR* mutations and (TG)m(T)n polymorphism in 112 Iranian CBAVD and 7 CUAVD males from Iran with 84 fertile males as control. Moreover, we compared the rate of decrease in exon 9+ transcripts with reduction of the (T) n repeat in our studied population. Our study showed that the *5T* mutation revealed a high frequency in our patients. The longer of (TG) m tract increases the proportion of exon 9– transcripts, but only when activated by the *5T* allele. The combination of the *5T* allele in one copy of the *CFTR* gene with a cystic fibrosis mutation in the other copy is the most common cause of CBAVD in Iranian population. We also observed the highest level of the exon 9+ splicing efficiency between the tested samples with the TG12T7 allele, which represents the most common IVS8 allele in the general population. Our results support the idea that a putative role of the (T)n repeat is to distance the TGm repeat from the 30 splice site, and the different alleles at the Tn locus affect the efficiency by which the splice acceptor consensus sequence is recognized.
Also This approach has allowed us to detect one novel nonsense mutation (K536X) in the NBD1 region and two novel missense mutations (Y122H & T338A) in the M2 and M6 regions of *CFTR* gene in our studied population witch were not reported previously. K536X nonsense mutation (transversion) was found in the first nuclear binding domain (NBF1), witch plays an important regulatory role in CFTR function therefore, considered as a sever allele responsible for elevated sweat test and obstructive azoospermia. Since Y122H and T338A mutations were compound heterozygote with the IVS8-*5T*, it is difficult to judge the severity of these mutations and their role in the CBAVD phenotype.

O-112: Application of Single-Needle Blastomere Biopsy in Human Preimplantation Genetic Diagnosis

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We have tested and subsequently successfully applied a single-needle approach to obtain blastomere biopsies from human preimplantation embryos for preimplantation genetic diagnosis (PGD). The method was first evaluated in a mouse system and shown to be compatible with a high degree of in vitro and in vivo development of biopsied mouse embryos. Furthermore, we showed that biopsied mouse embryos after transfer to recipient mice underwent implantation, normal development and delivery. Litters were followed through puberty and adulthood and shown to be normal with regard to sexual function and also a panel of biochemical and morphological parameters including organ histology. Successful human preimplantation diagnosis, followed by pregnancies and birth of healthy babies, was established with two out of three couples carrying a risk to transmit chromosomal abnormalities leading to severe disease. This is the first report of the successful use of a single-needle approach in human PGD. Considering its simplicity, we conclude that the single-needle approach is an attractive alternative for biopsies in PGD.

O-113: Recent Concern on Genetic of Infertility

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Modern genetics in general, as well as its clinical offshoot medical genetics, have gone through a huge development in the past decades. The progress achieved in elucidating the fine structure of the human genome is most impressive and occurs at an ever increasing pace. In reproductive medicine the application of modern DNA technology has already yielded a rich harvest. A considerable number of genes are now known that have an essential function in human reproduction and which, when deleted or mutated, can cause pathology in the male or female reproductive system.
Female and male factors can both contribute to infertility. Idiopathic infertility is a condition of couples unable to conceive for more than two years, with no abnormalities seen on repeated standard investigations for infertile couples, Among the major causes of infertility, chromosomal abnormalities, microdeletions and other genetic factors (FSH receptor mutation), in case of PCOs, endometriosis are important and have become more attention.
Infertility can occur either as an isolated disorder or within the framework of a known complex disorder or syndrome. In men, there is an excess of autosomal abnormalities in men with non-obstructive azoospermia or severe oligospermia. Besides, congenital bilateral absence of the vas deferens (CBAVD) associated with the phenotype of CFTR gene mutations cause obstructive azoospermia. There appears to be a world-wide concern over decreasing human sperm concentration but this has been highly controversial. Decreasing sperm counts are attributed to the deleterious effects of environmental contamination by heavy metals and estrogenic chemicals. To what extent there is a genetic contribution is unclear. Spermatogenesis is a complex process and it is subject to the influence of many genes; the molecular mechanisms involved are beginning to be understood. It is estimated that about 2,000 genes regulate spermatogenesis, most of these being present on the autosomes, with approximately 30 genes on the Y chromosome, Y genes are not essential for general body function except with regard to vital male reproductive processes. Recent advanced development in molecular genetics over the past decades, caused a significant proportion of idiopathic male infertility in otherwise healthy males is now known to be geneticin origin. Our knowledge of the molecular genetics of human fertility is expanding rapidly. It will discuss more in detail about impairment of genetic causes of different stage of ART treatment cycles as well.

O-114: Preimplantation Genetic Screening (PGS)

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PGS is a procedure that allows us, during IVF treatment, to select the best embryos before transfer not only according to their morphology and cell number but also on the basis of the number of chromosomes. PGS should only be used at the moment in a selected group of patients who make a lot of chromosomal abnormal embryos: 1. patients who are older than 35–37 years; 2. patients who had already several failed IVF attempts; 3. patients who had three or more recurrent miscarriages and last but not least couples where the male partner has a non-obstructive azoospermia. A summary of the world literature and our own data will be presented. There is a strong feeling that PGS will give some extra information for the selection of the best embryos in this group of patients. However, it would be premature to jump to conclusions about the possible advantages of PGS without clinical randomized studies.

O-115: The Murine Dnali1 Gene Encodes a Flagellar Protein that Interacts with the Cytoplasmic Dynein Heavy Chain 1

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Introduction: Axonemal dyneins are large motor complexes generating the force for the movement of eukaryotic cilia and flagella, whose functional disruption leads to loss of motility and may result in male infertility or lateralization defects. Axonemal dynein motors can be divided into outer and inner arms and are best characterized in the Chlamydomonas. In the present study, the molecular analysis and identification of p28 orthologous gene Dnali1 (dynein axonemal light intermediate chain) is reported.
Materials and Methods: A Dnali1 genomic clone was obtained from RZPD, restriction analyzed and verified by southern blot analysis. The clone was biotinylated and hybridized to find its chromosomal localization. Polyclonal antibodies were generated and expression analysis was performed by immunohistochemistry and western blotting. Also, RT-PCR and northern blot experiments were performed. To study the interaction partners, Yeast two-hybrid screening, coimmunoprecipitation and colocalization assays were performed. **Results:** Dnali1 gene comprises six exons, located on mouse chromosome 4 and is expressed ubiquitously by RT-PCR. By Northern blot analysis, two Dnali1 specific transcripts are detected in the testis. Dnali1 is localized along the entire axoneme and is strongly expressed in spermatids. By yeast-two-hybrid screen and coimmunoprecipitation assay, Dnali1 is identified to interact with the C-terminus of cytoplasmic dynein heavy chain (Dnchc1). Indirect immunofluorescence also demonstrates a coincided colocalization of Dnali1-myc and Dnchc1-E2. Taken together, the results verify that Dnali1 interacts with Dnchc1 within the male germ cells. **Conclusion:** Dnali1 localization pattern suggests that it is an integral component of axoneme and plays a crucial role in spermatogenesis. The precise role of Dnali1 in the ciliary/flagellar motility is yet unknown, however, an increased expression level in males containing post-meiotic germ cells supports the role of Dnali1 in spermatid differentiation. Dnali1 expression in the tissues and cells having no axoneme and interaction with Dnchc1 suggests that it may have multiple roles. It is unclear whether Dnali1 is a component of the cytoplasmic complex. Our data reveals that DNALI1 is not a possible candidate gene for primary ciliary dyskenisia (PCD) syndrome.

O-116: High Resolution Mapping of Ribosomal DNA in Early Mouse Embryos by Fluorescence *in Situ* Hybridization

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Introduction: The nucleolar precursor bodies (NPBs) are numerous discrete entities present in the nuclei of early mammalian embryos. So far, agreement seems to exist about participation of NPBs in the assembly of the mature nucleolus and its implication in the structural support of reactivated rRNA genes (rDNA). However, whether all rRNA genes, including those not transcribed, are spatially associated with NPBs and what is the general arrangement of rDNA in early mouse embryos are unanswered questions so far.
Materials and Methods: We examined the localization of rDNA in transcriptionally silent (one-cell and early two-cell) and transcriptionally competent (late two-cell) mouse embryos by an original protocol for a highly sensitive fluorescence *in situ* hybridization with probes complementary to mouse rDNA repeats.
Results and Conclusion: Our results showed that irrespective of the rDNA transcriptional status, one or more NPBs per nucleus were not structurally associated with rDNA. These observations support the idea that NPBs are heterogeneous in their ability to recruit rRNA genes and thus to participate in reassembly of the mature nucleolus. As in somatic cells, and despite the absence of the characteristic nucleoli, the general arrangement of rRNA genes in early mouse embryos reflected the intensity of rDNA transcription. rRNA genes were unequally distributed between nucleolar organizing region (NOR)-bearing chromosomes at the first cleavage division, and more strikingly, between sister chromatid NORs of a single nucleolar organizing chromosome. The latter indicates that sister chromatids might harbor various numbers of the rRNA gene copies, and that the genes might be unequally distributed between the two blastomeres during the first mitosis.

O-117: Functional Genomics of Stem cell Therapy

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Stem Cells

O-118: Cancer Stem Cell

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Cancer is a multi-factorial disease which can originate from any tissue in the body. Cancer cells resemble stem cells in many biological properties: they can self renew and they can differentiate to more mature cells (observed in well differentiated cancers).
According to this model ,tumors may be regarded as abnormal organ that contain a hierarchy of cells including self-renewing stem calls and highly proliferative progenitor cells that , in turn ,give rise to differentiated cells comprising the bulk of an organ or tumor.
Normal stem cells proliferation tightly controlled by microenvironment or nich. Disruption of this control may deregulate stem cell proliferation and carcinogenesis.
First evidence for presence of cancer stem cells observed in leukemic cells transplanted to immunodefficient mice. Only a subpopulation of leukemic cells can produce leukemic phenotype in transplanted mice so it seems that every leukemic cells are not biologically equal and only a minority of these cells can maintain leukemia.
Similar experiences in other malignancies such as breast cancer, brain tumors of children and colon cancer consolidate this hypothesis and many researches are ongoing for better defining of characterization of these cells in each malignant tumors.
Cancer stem cell hypothesis has important implications for cancer prevention and treatment.
Current protocols of cancer treatment only attack to bulk of tumor calls that comprise

most of tumor cells or eradicate highly proliferating cancer cells.
Cancer stem cells are minority of caner cells and usually are in dormant state so current therapies can not eradicate cancer stem cells. So despite of early success of cancer control but eventually we will observe caner recurrence and progress.
So characterization and better understanding of these cells could help us for better knowledge of cancer biology, behavior and also better prediction of cancer prognosis and finally better treatment.

O-119: Pluripotent Stem Cell Epigenetics during Development and Cancer

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In the widest sense, epigenetic alterations are functional modifications of DNA by external conditions, leading to the modulation of gene expression without altering DNA sequences. The three main types of molecular epigenetic changes are genomic imprinting, DNA methylation and histone modification by acetylation, methylation and phosphorylation. Pluripotent stem cell proliferation, differentiation and apoptosis during development and cancer constitute one of the best models to study this phenomenon. The precise timing of imprint erasure and re-establishment for many genes remains to be determined and the precise molecular mechanisms of genomic imprinting have not yet been fully characterized. In this regard, we have analyzed the methylation state and DNase-I sensitivity of two genes with opposite genomic imprinting (U2af1-rs1and H19) in pluripotent germinal cells (EG1), isolated post-natal spermatogonia and mature sperm cells. We also studied the epigenetic regulation of imprinting genes during embryonic stem cell differentiation by retinoic acid and the involvement of epigenetic modifications in the apoptosis of these cells. We are currently examining the involvement of matrix metalloproteinases, insulin-like growth factors, transforming growth factor beta, cadherin/catenin complex and integrins in the processes of invasion and differentiation in embryonal carcinoma cells.

O-120: Generate Retinal Cells from Retinal Stem Cells to Repair the Retina

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The two laste years we have isolated and characterized retinal stem cells from the newborn mouse retina to generate retinal cells in vitro. Using different protocols, we are able to generate the retinal ganglion cells, which connect the retina to the brain and the photoreceptors. Transplantation of retinal stem cells in degenerated retina leads to the generation and integration of retinal ganglion cells, which could be an interested cell source for different forms of glaucoma. In parallel, we studied the potential of cells commited towards the photoreceptor fate to integrate a diseased retina. We observed a start of photoreceptor differetiation and cell integration, but the newly generated photoreceptors did not survived. These data show that it is possible to generate adequate cells to repair the retina, but that environmental factors sustaining the survival of the transplanted cells have to be identified to obtain an efficient retinal repair.

O-121: Keratolimbal Allograft Surgery

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Objective: Evaluation of anatomical results and visual outcomes of keratolimbal allograft surgery.

Materials and Methods: patients with total limbal stem cell deficiency (comfirmed by impression cytology) and adequate tear production (schirmer test>10 mm) were included. Keratolimbal allograft surgery was performed in standard fashion. Main outcome measures were improvement of vision and corneal epithelialization. Immunosuppression using mycophenolate and cyclosporine was done.
Results: Eleven eyes of 10 patients (9 female, 1 male) were operated. Primary diagnosis waschemical burn (n=8) and SJS (n-2). Age range was 6-32 years. Follow up range was 69-416 days. Visual acuity improved in 7 eyes, and was unchanged in 4 (1 due to primary failure and in 3 cases due to severe corneal opacity). Cornea was completely epithelialized in 6 eyes during 1 week. Epithelial failure was secondary to primary failure (n=2) and exposure (n-1). Penetrating keratoplasty was done in 3 cases. Their vision was 20/50 in 2 eyes 20/40 and in one. Deep anterior lamellar keratoplasty was done in 1 eye. KLAL rejection occurred in 2 eyes which controlled with high dose steroid therapy. Re-KLAL was done in 1 eye which was failed due to surface problems. Sector KLAL was done 2 times in 1 eye due to sectoral melting associated with limbal ischemia and chronic exposure.
Conclusion: Kertolimbal allograft surgery is successful in visual rehabilitation of patients with total stem cell deficiency.

O-122: Transcriptional Profiling of Mammary Gland Side Population Cells Reveals New Roles for this Putative Mammary Gland Stem Cell Sub-Population

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Mammary gland (MG) contains a distinct population of Hoechst-effluxing side population cells (SPs), known as MG-SPs. Bone marrow SP have been shown to be enriched in hematopoietic stem cell activity. However, evidence that MG-SPs are enriched for functional epithelial stem cell activity is only correlative. To better characterize MG-SPs, their microarray gene profiles were compared to the remaining cells, which retain Hoechst dye (MG non-SP [MG-NSP]). For analysis, Gene Ontology (GO) that describes genes in terms of biological processes and Ontology Traverser (OT) that performs enrichment statistics were used. OT showed that MG-SP-specific genes were enriched in GO categories of organogenesis, vasculogenesis, cell cycle regulation and checkpoints, and multidrug resistant transporters (MDRs). The MG-NSP-upregulated genes were enriched in GO category of cellular organization, including mammary epithelial specific genes, p63, smooth muscle actin, myosin, -6 integrin, cytokeratin (CK) 14, CK8 and CD24. The higher expression of endothelial specific genes such as angiogenic ligand, tanscritption factors and progenitor markers and markedly lower expression of epithelial specific genes suggest that MG-SPs may represent MG endothelial stem cells. There is increasing evidence that direct interactions between endothelial and epithelial stem cells play a significant role in tissue development and tumorigenesis. We speculate that MGSPs, through direct interactions with mammary epithelial stem cells, may participate in mammary development and tumorigeneis. Our gene profiling has allowed the identification of a novel surface marker, endothelial specific adhesion molecule (ESAM), exclusively expressed by MG-SPs. The identification of ESAM will facilitate functional studies of MG-SPs, which has previously been difficult due to the toxicity of Hoechst dye used to isolate these cells. In conclusion, gene profiling of MG-SPs has shed new light on their identity. These studies have the promise to open new avenues for the development of novel anti-cancer therapeutic strategies.

O-123: Human Embryonic Stem Cells: The Mother of All Cells

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Rapid advances in the field of assisted conception have led to the isolation and growth of human embryonic stem cells (hESCs) from 5 day old embryos left over from In Vitro Fertilization (IVF) programs. These cells are very versatile and can be coaxed into almost all the tissues types of the human body and therefore offers promise in the treatment of a variety of incurable diseases by transplantation and gene therapy, provide an ideal screening tool for potential drugs in the pharmaceutical industry and allows the study of early human development and what goes wrong in infant cancers. All NIH registered hESC lines have been derived and propagated on mouse feeder cells thus limiting their clinical application for fear of transmission of unwanted agents from mouse to human cells. Clinical grade hESC lines derived and propagated in xeno-free culture conditions and under cGMP facilities are now available. Other important studies such as the unraveling of the genetic secrets driving these cells into various tissues are also being undertaken and such results will be useful in the development of proteins and growth factors that could help to produce stable tissues from hESCs. The derivation, propagation, behaviour, storage, genomics and nature of these mysterious cells will be presented.

O-124: Stem Cells: From Bench to Bedside

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Stem cell biology has attracted tremendous interest recently. Several varieties of stem cells have been isolated and identified and can be broadly classified into embryonic, fetal and adult stem cells. Their versatility ranges from unipotentiality to multipotentiality to pluripotentiality. Human embryonic stem cells appear to be the most pluripotent but their clinical application appears to be fraught with obstacles such as the production of benign teratomas and possible rejection of hESC-derived tissues during transplantation therapy. Studies are being undertaken to overcome these hurdles and the approaches are encouraging. Several groups have been successful in directing hESCs via spontaneous, chemical, genomic and coculture methods into desirable cells and tissues such as pancreatic islets, neurons, heart cells and blood cells for future treatment of diabetes, neuronal, cardiovascular and blood borne diseases respectively by cell based transplantation therapy. The application of these derived tissues is being validated in animal models. Work is in progress with respect to dosage, route of administration, stability and duration of functional competence of transplanted cells. Progress is also being made on the isolation and differentiation of adult mesenchymal, bone marrow and umbilical cord stem cells for future cell based therapies. The background to all these studies will be presented.

O-125: Peripheral Blood Hematopoietic Stem Cell Transplant in Malignant Lymphoma Biology, Clinical Indications and Results

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Hemopoietic progenitor cells can be mobilized from bone marrow into peripheral blood. This was initially observed during recovery after myelosuppressive chemotherapy and the ability of recombinant hematopoietic growth factors to mobilize blood cells, either alone or by enhancing the effect of chemotherapy, facilitated the use of peripheral blood hemopoietic progenitor cells (PBPC) for transplantation. The lecture will illustrate the biological bases of hematopoietic progenitor cell mobilization, together with the principal mobilization protocols; the factors affecting yield and the type of cells mobilized will be

discussed, as well. The clinical applications of peripheral blood hemopoietic stem cells will be illustrated, with particular emphasis on their use for the hematological rescue after high-dose chemotherapy in malignant lymphoma and multiple myeloma. The most important clinical trials in this field will be presented, with emphasis on the results of autologous PBPC tranplantation as salvage therapy of malignant lymphoma (either Hodgkin or non-Hodgkin). The prospective utility of autologous PBPC transplantation, combined with immunotherapy, as front line therapy in aggressive and follicular lymphoma will be discussed. Data on the use of PBPC in an allogeneic transplantation setting after non-myeloablative conditioning chemotherapy will be presented, as well.

O-126: Is there a Place for Peripheral Blood Hematopoietic Stem Cell Transplant in Solid Tumors?

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Autologous peripheral blood hematopoietic stem cell (PBSC) transplantation is extensively utilized in malignant lymphoma (either Hodgkin or non-Hodgkin) and is being recognized as standard therapy for relapsing or resistant disease. This procedure is also applied in multiple myeloma and in non-neoplastic conditions such as systemic amyloidosis and autoimmune diseases (in controlled clinical trials, only). Because of their chemosensitivity, a clinical utility for this procedure has been envisaged in breast and ovarian cancers, in germinal-cell tumors and in neuroblastoma. The lecture will review this topic and deal mostly with clinical data obtained with autologous PBSC transplantation in the adjuvant setting of high-risk breast cancer (ten or more positive axillary nodes). Randomized studies comparing high-dose chemotherapy followed by autologous hemopoietic stem cell transplantation versus conventional-dose chemotherapy have yet reported somehow discordant results; a tentative explanation for this discordance will be provided. A novel promising approach consists of allogeneic PBSC tranplantation after non-myeloablative conditioning regimens (reduced intensity chemotherapy or RIC). This procedure couples the anti-tumor effect of chemotherapy with the adoptive immunologic effect of an hypothetical graft-versus-tumor reaction and is being experimentally evaluated in chemosensitive tumors and in those responsive to immunotherapy. The results obtained, so far, with this experimental approach will be discussed.

O-127: Role of Notch Signaling in Normal and Neoplastic Hematopoietic Stem Cells, and Clinical Application of Notch Signal Modifiers

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The Notch receptor signaling pathway is among the most commonly used communication channels in animal cells. One main focus in mammalian studies has been the role of Notch in stem cell systems in embryonic and post-embryonic states. I have been studying the role of Notch signaling system in normal and neoplastic hematopoietic cells, as well as biochemically characterizing the interaction between four Notch receptors and their physiological ligands. Major findings include identification of the *Notch1* gene being essential for hematopoietic stem cell generation and the

master hematopoietic stem cell transcription factor,Runx1/AML1, as the regulator that functions downstream of Notch1 signaling during embryogenesis, identification of the *Notch2* gene being essential for a B cell subset generation from hematopoietic stem cells, discovery of oncogenic mutations of *Notch1* gene in T cell acute lymphoblastic leukemia (T-ALL) cells and *Notch2*gene in B cell non-Hodgkin lymphoma cells, demonstration of *in vivo* efficacy of a new γ-secretase inhibitor for TALL, and demonstration of highly efficient *ex vivo* expansion of human cord blood hematopoietic stem cells by the use of a soluble form of Notch ligand, the Delta1-Fc chimeric protein. In the last subject, we showed 5.8-fold actual expansion of long-term SCID-repopulating cells, the golden standard for the human hematopoietic stem cell measure, in a chemically defined culture condition without serum. Based on this result, I plan to set a clinical trial in which hematopoietic stem cells from one unit of cord blood are expanded and transplanted together with another cord blood unit to improve the outcome of the cord blood transplantation.

O-128: Transplantation of Undifferentiated Mesenchymal Stem Cell in Distraction Osteogenesis in a Canine Model: A Histological and Histomorphometrical Study

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Objective: The purpose of this investigation was to study the effect of Mesenchymal Stem Cells (MSCs) on early bony consolidation in distraction osteogenesis in a dog model.
Materials and Methods: In this study, 10 adult male dogs (15-25 kg) were used. The dogs were randomized into two groups, each consisting of five animals. Lengthening of 30 % of the left tibia was performed in each dog by callus distraction after osteotomy and application of an Ilizarov fixator. Distraction was started at the seventh postoperative day with a distraction rate of 0.5 mm twice a day. Culture-expanded autologous MSCs and platelet-rich plasma (PRP) (MSCs/PRP group) and PRP (PRP group) were injected into distracted callus at the middle and the end of the distraction periods (each time 1×10^7 passage-4 cells). At the end of distraction period, Ilizarov fixator was replaced by interlocking nail to allow consolidation periods as twice the lengthening period. The dogs were killed at the end of the consolidation phase. The tibia was removed from the distracted left leg and after tissue processing longitudinal sections were cut and stained with Hematoxyline & Eosin and Trichrome Masson. In all tibiae, the bone regeneration was evaluated histomorphometrically with an image analyzing software and the results were compared between two groups.
Results: Histologically, the area of new bone trabeculation was more advanced for the MSCs/PRP group, with less intervening fibrovascular intermediate zone in the bony regenerate. The amount of newly formed bone in the MSCs/PRP group was significantly greater than in the PRP group.
Conclusion: These findings suggest that MSCs seems to be very effective in early bony consolidation in distraction osteogenesis. The use of MSCs may allow a shortened period of consolidation and therefore permit earlier device removal.

O-129: Culture of Spermatogonial Stem Cells

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The possibility to culture and propagate spermatogonial stem cells (SSC) in vitro would enable the development of a protocol through which testicular biopsies could be taken from young cancer patients before chemotherapeutic treatment, SSC would then be propagated in vitro, cryopreserved and transplanted back to the patients testis after cure. In addition, the access to sufficient numbers of spermatogonial stem cells (SSC) would allow detailed studies of their regulation and further biomanipulation. We presently work on bovine and human SSC, trying to develop protocols to culture these cells. For bovine SSC a specialized medium and several growth factors were tested to study the in vitro behavior of bovine type A spermatogonia, a cell population that includes the SSC. During culture, colonies appeared the morphology of which varied with the specific growth factor(s) added. Whenever stem cell medium was used, within two weeks of culture seminiferous tubule-like-structures appeared in the core of the colonies. Remarkably, the tubule-like-structures always contained A spermatogonia. When LIF, EGF or FGF2 were added, specific effects on the numbers and arrangement of somatic cells were observed. However, the number of A spermatogonia was significantly higher in cultures to which GDNF was added and highest when GDNF, LIF, EGF and FGF2 were all present. The latter suggests that a proper stimulation of the somatic cells is necessary for optimal stimulation of the germ cells in culture. Somatic cells present in the colonies included Sertoli cells and peritubular myoid cells. A transplantation experiment, using nude mice, showed the presence of SSC before and after culture and in addition strongly suggested a more than 100 fold increase in the number of SSC after 30 days of culture. These results demonstrate that bovine SSC self-renew in our specialized bovine culture system and that this system can be used for the propagation of these cells. Prelimenary results with human SSC also indicate the formation of colonies containing spermatogonia, likely including SSC, that can be passaged multiple times. In both bovine and human cultures aberrant colonies were observed regularly. These aberrant colonies are now studied in more detail as they might represent ES-like cell colonies that in the mouse were found to be derived from cultured SSC.

O-130: Spermatogonial Stem Cells: Effects of Shorttime Exposure to High Temperature

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In human male infertility patients spermatogenesis can be found to be disturbed in various ways. First, spermatogonia and spermatogonial stem cells may have been killed by chemotherapeutic drugs or irradiation. In rodent models it was found that in general surviving stem cells will be induced to repopulate the seminiferous epithelium by enhanced proliferation and selfrenewal first and subsequent normal differentiation leading to normal spermatogenesis in the recovered areas. Second, in the previous case in the rodent model, differentiation of early spermatogonia may become arrested because of too high testosterone levels. Third, a mutation may be present causing arrest at a specific step of the spermatogenic process. In rodents many examples for this were found causing arrests at many steps of the spermatogenic process. We now have found a fourth possibility in rats exposed either to artificial hemicryptorchidism lasting 48 hr, or to heat treatment of both testicles to 43^0C for 30 min. Already, 3 weeks after treatment there was a 40% decrease in the weight of the previously cryptorchid testes, and 65% in the heat treated testes. Remarkably, between 3 weeks and 175 days there was no recovery. In sections of testes at 175 days, in the previously cryptorchid testes there was a mixture of tubules with normal full spermatogenesis and tubules with only very few germ cells. On the average 73% of the tubules showed full spermatogenesis but in the rest, spermatogenesis was severely compromised. The short time period of

heating of the testis appeared to have even more severe longterm effects than two days of cryptorchidism. In these rats, some seminiferous tubules with full spermatogenesis were found in the testes of only one of 3 rats. Interestingly, the morphological picture of spermatogenesis in the affected tubules in previously cryptorchid rat testes and heat treated rat testes was comparable. In the affected tubules in both situations on the average about 30% of the tubule cross sections contained no germ cells, while the most advanced cells were A spermatogonia in another 30%, B spermatogonia or preleptotene spermatocytes in 20% and in 20% of the tubules more advanced cells than preleptotenes were seen, including some occasional spermatids. These data indicate that there was not an arrest in the differentiation of the germ cells. Apparently, the effects of a shortterm cryptorchidism or heat treatment do not evoke a recovery reaction, for example by way of enhanced self-renewal and proliferation of stem cells as occurs shortly after irradiation. The effects of shortterm high testicular temperatures are reminiscent of what can regularly be seen in testis biopsies of infertile human patients. Tubules showing only Sertoli cells or poor spermatogenesis together with an island of tubules with normal spermatogenesis are not uncommon. The possibility of heat-induced damage in these cases should be studied. Furthermore, shortterm cryptorchid rats and rats which received a shortterm testicular heat treatment may be a good model to find ways to stimulate spermatogenesis when the epithelium itself does not initiate recovery mechanisms.

O-131: Microanatomical Study of Effects of Mesenchymal Stem Cells on Striatal Lesion by Quinolinic Acid

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Introduction: Huntington's disease is an incurable disease happens in adults. This disease inherited neurodegenerative disorder caused by repeating of CAG bases in 5' coding region of HD gene (coding Huntington protein, expressed in all tissues). In advanced form of disease, progressive striatum atrophy happens and medium spiny neurons are dead. These neurons comprise more than 80% of striatum neurons in adults. By passing time, also new cortex and other regions of the brain show atrophy. Nowadays, there is no effective therapeutic strategy for diminishing the motor disorder of Huntington's disease. In recent years, cellular transplantation has been an effective therapeutic method for neurodegenerative diseases.
Materials and Methods: In this paper, the effects of bone marrow derived mesenchymal stem cell were assessed in animal model of Huntington's disease. After causing unilateral lesion in striatum by Quinolinic acid, bone marrow derived mesenchymal stem cells which had been isolated and purified from 4-6 weeks old rats, transplanted into the damaged striatum. After nine weeks of transplantation, the volume of striatum, lateral ventricle and hemispheres was measured in control and test (experimental) groups. After volume determination, the atrophy percentage of both striatum and damaged hemisphere and volume extension of lateral ventricle was calculated.
Results: Histological results showed significant difference in volume and percentage of striatum atrophy between sham and test groups. This assay suggests using of bone marrow derived mesenchymal stem cells in impaired of motor disorders of Huntington's disease as a novel out look of their multipotency.
Conclusion: According to results of this investigation, cell therapy by means of bone marrow derived adult stem cells promises for treatment of neurodegenerative disease, especially Huntington's disease.

O-132: Polyamines Modulate Nitric Oxide Production and *Cox-2* Gene Expression in Response to Mechanical Loading in Human Adipose Tissue-Derived Mesenchymal Stem Cells

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For bone tissue engineering, it is important that mesenchymal stem cells (MSCs) display a bone cell-like response to mechanical loading. We have shown earlier that this response includes increased nitric oxide (NO) production and cyclooxygenase-2 (*COX-2*) gene expression, both of which are intimately involved in mechanical adaptation of bone. *COX-2* gene expression is likely regulated by polyamines, which are organic cations implicated in cell proliferation and differentiation. This has led to the hypothesis that polyamines may play a role in the response of adipose tissue-derived MSCs (AT-MSCs) to mechanicalloading. The aim of this study was to investigate whether genes involved in polyamine metabolism are regulated by mechanical loading and to study whether polyamines modulate mechanical loading-induced NO production and *COX-2* gene expression in human AT-MSCs. Human AT-MSCs displayed a bone cell-like response to mechanical loading applied by pulsating fluid flow (PFF), as demonstrated by increased NO production and increased gene expression of *COX-2*. Furthermore, PFF increased gene expression of spermidine/ spermine *N* (1) - acetyltransferase, which is involved in polyamine catabolism, suggesting that mechanical loading modulates polyamine levels. Finally, the polyamine spermine was shown to inhibit both PFF-induced NO production and *COX-2* gene expression, suggesting that polyamines modulate the response of human AT-MSCs to mechanical loading. In conclusion, this is the first study implicating polyamines in the response of human AT-MSCs to mechanical loading, creating opportunities for the use of polyamines in tissue engineering approaches targeting skeletal defects. STEM CELLS

O-133: Exploiting Proteomics for Stem Cell Research

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The application of proteomics is beginning to have an impact, enhancing our understanding of stem cells biology. We have applied proteomic analysis to identify signaling pathways and molecular mechanisms involved in the maintenance of self-renewal, pluripotency, and/or multipotency of stem cells. Analysis has currently been applied to total, nuclear and membrane proteomes as well as phosphoproteome of differentiated and undifferentiated human, mouse and monkey embryonic stem cells. Owing to comparative analyses, several proteins and mechanisms emerged as key participants in stem cells proliferation and differentiation. The application of proteomics is greatly being assisted by links to gene manipulation, epigenomics and transcriptomics.

O-134: Assessment of the Cationic Colloidal Silica Method of Plasma Membrane Enrichment for Use in Proteomic-Based Discovery of Cell Surface Biomarkers

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Characterisation of differences between cancerous and normal tissues is the first step

towards developing diagnostic tests and targeted therapeutics to combat cancer. The appeal of plasma membrane proteins as disease biomarkers is their accessibility, due to their expression at the cell surface and potential to be shed into the bloodstream, which allows both their detection in diagnosis and use as therapeutic targets.
Classically, integral membrane proteins have been difficult to detect using 2D-PAGE technology due to their low relative abundance, their hydrophobicity, and the variability of their post translational modifications. In order to overcome the abundance issue, several methods have been used to enrich the cell surface subproteome, including density gradient centrifugation, biotinylation and density perturbation methods. Herein, we have assessed one of the density perturbation methods, the cationic colloidal silica method, for enriching membrane proteins from a classical Ras transformation model. After enrichment of the plasma membrane, SDS-PAGE was used to separate the proteins, to maximize membrane protein solubility and recovery, prior to in-gel tryptic digestion and LC-MS/MS to identify the isolated proteins. This comprehensive analysis of 61 discrete gel slices yielded 261 manually validated, high confidence protein identifications. The size-based fractionation of SDS-PAGE allowed further validation of the MS/MS identifications by comparing their predicted molecular weight based on primary sequence and their relative migration in the gel. Using subcellular localization information showed a number of integral and associated membrane proteins were identified, however, several proteins from other cellular organelles were present, particularly nuclear and ribosomal proteins. Proteins identified from these organelles showed a bias for extremely basic isoelectric points, compared with proteins identified from other locations, indicating the cationic colloidal silica method also enriches basic proteins due to the ionic nature of the preparation.

O-135: 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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Introduction: 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 grafted cells in improvement of spatial memory function were investigated.
Materials and Methods: 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. 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.
Results: 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.
Conclusion: 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.

O-136: Human Embryonic Stem Cells, Actual Applications and Challenges

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Human embryonic stem (hES) cells are pluripotent cells derived from the inner cell mass cells of blastocysts. hES cells are important research tools in studies of the physiology of early tissue differentiation. In addition, prospects are high regarding the use of these cells for successful cell transplantation for the treatment of various human diseases. However, there are several challenges to overcome before these cells could be used in a therapeutic manner. One concern has been that cultivation of these cells over many passages might induce chromosomal changes. It is thus important to investigate these cell lines, and check that a normal chromosomal content is retained even during long-term in vitro culture. Others factors which may limit the therapeutics application of hES cells: are continuous culture of hES cells in an undifferentiated state that requires the presence of feeder layers and animal-based ingredients which represent a risk of cross-transfer of pathogens. In addition differentiated hES cells express molecules which could cause immune rejection.
We have gradually improved our hESC derivations. Human skin fibroblasts were used as feeder cells in derivation of all our 25 permanent fully characterised hESC lines. In the first four derivations, foetal calf serum (FCS) was used as a supplement in the medium, Thereafter, serum replacement (SR) medium was used. Immunosurgery generally used for isolation of the inner cell mass (ICM) still involves animal serum and complement.
We developed a practical mechanical isolation method for the ICM. Two flexible metal needles with sharpened tips, 0.125 mm in diameter, were used to open the zona and extract the ICM under a stereo microscope. The technique is fast, and does not require any extra investment. Mechanical isolation of the ICM proved to be an effective way to derive new hESC lines. The xeno-components of immunosurgery could be avoided.

O-137: *In Vivo* Neurogenesis Potentials of Adult Mesenchymal Stem Cells

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Introduction: There is increasing evidence that mesenchymal stem cells (MSCs) isolated from the bone marrow or adipose tissue are multipotent cells that can engraft and differentiate in the CNS. MSCs may offer advantages if developed for cell therapy because these cells are relatively easy to isolate from small volume of lipoaspirate or of bone marrow aspirate and can be readily expanded to provide a sufficient number of cells for transplantation. In this study, a series of CNS transplantation experiments were performed to characterize the engraftment, migration, homing, and differentiation patterns of human MSCs.
Materials and Methods: Cell lines generated from human bone marrow derived MSCs (BMSCs) and adipose tissue derived MSC (ASCs) were injected into lateral ventricle of brain of NIHIII immune deficient mice by stereotaxic injection. The fate of MSCs was monitored by immunohistochemistry and PCR analyses 15 and 30 days post-injection.
Results and Conclusion: Engrafted MSCs migrated along ventricular area both rostrally and caudally from the injection site. Cells were distributed throughout the entire rostro-caudal extent of ventricular zone. Many MSCs also were detected lining the ependyma throughout the ventricle. A significant number of donor cells were distributed along the length of the spinal cord. A higher frequency of MSCs was detected in animals injected with ASCs than BMSCs at 15 days following injection. Donor cells were dispersed into both lobes of the brain, numerous cells were detected in the cortex and different areas of cerebrum such as semilunar lobules, paraflocculus, postsuperior, fissure areas, and external and internal granular layers. Detailed analysis revealed that MSCs were predominantly seen in perivascular space in the brain. In animals injected with ASCs, the total number of detectable cells was significantly higher in 30 days post-engraftment than 15 days, though the distribution pattern was unchanged. A significantly higher number of engrafted BMSCs were detected in the spinal cord of 30 days group. BMSCs were widely distributed throughout the brain at both time points. The distribution pattern of BMSCs at 15 days post engraftment was similar to ASCs. At 30 days, the majority of cells were found in the ventral areas of brain, with many detected in the immediate periphery of basilary artery. The results form these studies suggest that the MSCS effectively migrate throughout the CNS via the cerebrospinal fluid, as the pattern of detection of cells parallels the flow of cerebrospinal fluid in the brain. Numerous MSCs were found to be reactive to some specific neuronal antibodies such as NSE and MAP-2 at 30 days following engraftment indicating the differentiation of the grafted cells. The results of these studies demonstrated that transplantation of both types of MSCs into the ventricle resulted in the migration of MSCs throughout the brain within striatal, cortical, and cerebellar regions as well as and spinal cord.

O-138: Stem Cells, Pluripotency and Epigenetic Reprogramming

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One of the key issues raised by nuclear cloning is the question of genomic reprogramming, i.e. the mechanism of resetting the epigenetic modifications that are characteristics of the adult donor nucleus to

ones that are appropriate for an embryonic cell. The mechanisms by which embryonic stem (ES) cells self-renew while maintaining the ability to differentiate into virtually all adult cell types are not well understood.
To establish a core transcriptional circuitry of pluripotency and self renewal we have previously identified Oct4, Sox2, and Nanog target genes using genome-scale location analysis. We found that Oct4, Sox2, and Nanog co-occupy a substantial portion of their target genes that are either active or repressed in embryonic stem cells. These repressed target genes frequently encode transcription factors, many of which are developmentally important homeodomain proteins. To gain insights into the role of these trasncitpiotn factors in gene repression we have studied the role of PcG proteins in both human and murine ES cells using genome-wide location analysis. PcG proteins similarly co-occupied a large cohort of genes in both human and murine ES cells, many of which encode transcription factors with important roles in development. The transcription factors Oct4, Sox2, and Nanog are associated with a subset of developmental regulators that are repressed in human ES cells. Interestingly, each of the three DNA-binding transcription factors occupied approximately one-third of the PcG-occupied developmental genes, supporting a link between repression of developmental regulators and stem cell pluripotency. These data provide insights into mechanisms by which pluripotent cells may be stimulated to differentiate into different cell types or by which somatic cells might be reprogrammed back to the pluripotent state.

O-139: *In Vitro* Reprogramming of Fibroblasts into a Pluripotent ES Cell-Like State

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The full term development of sheep, cows, goats, pigs and mice has been achieved through the transfer of somatic cell nuclei into enucleated oocytes. Despite these successes, mammalian cloning remains an inefficient process, with a preponderance of reconstructed embryos failing at early to midgestation stages of development. The small fraction of conceptuses that survive to term are characterized by a high mortality rate and frequently display grossly increased placental and birth weights. It is likely that inappropriate expression of crucial developmental genes may contribute to lethality of cloned embryos. One potential use of the nuclear cloning approach is the derivation of “customized” ES cells for patient specific cell treatment but technical and ethical considerations impede the therapeutic application of this technology. Therefore, attempts have been made to generate pluripotent ES cell-like cells *in vitro* without the use of eggs.
Reprogramming of fibroblasts to a pluripotent state can be induced *in vitro* through ectopic expression of the four transcription factors Oct4, Sox2, c-Myc and Klf4. Our results show that DNA methylation, gene expression and chromatin state of such induced reprogrammed cells are similar to that of ES cells. Importantly, the cells can form viable chimeras, can contribute to the germ line and can generate live late-term embryos when injected into tetraploid blastocysts. These data demonstrate that the biological potency and epigenetic state of *in vitro* reprogrammed iPS cells are indistinguishable from that of ES cells. The implications of these results for transplantation medicine will be discussed.

O-140: Mesothelial Stem/ Progenitor Cells of the Peritoneum Clonal Characterization, Isolation and Differentiation

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Introduction: 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 reviewd in previous works. Mesothelial cell have been reported to share tumour and hematopoietic cell lineage markers, implying a common developmental background of hematopoeitic and mesothelial cells.

Materials and Methods: In order to investigate the potential plasticity of mesothelial cells, we characterized cell populations of the mesothelium within the peritoneal dialysis liquid from early stage non peritonitis patients by immunostaining for CD34, HBME-1, CD44, CD31, WT-1,CD38 markers, having had tracked and characterized mesothelial cells post transplantation in mice recipient tissues by FISH beforehand (Moallem et al, 2007).We isolated and analyzed mesothelial clonal populations that expressed CD34, HBME-1 by means of standard clonogenic procedures and consequently by FACS (Fluorescent activated cell sorter). Differentiation of the isolated colonies was induced toward neurons,skeletal muscle and osteoblsts an verified by morphology and histology studies, immunostaining, RT-PCR and western blotting.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 neurofilamenet 200, Nestin, Tubulin B expression, Differentiation towards skeletal muscle was induced by TGF-B and Dexamethasone. Muscular identity of the cells was testified by the expression of troponin1 skeletal muscle, myosin heavy chain. Differentiation towards osteoblasts was induced by B-glycerophosphate, TGF-B and Dexamethasone. Osteocalcin, oil red and ALP staining was accomplished.

Results and Conclusion: Screening cell populations of the peritoneum, revealed the dominance of HBME-1 and CD34 markers. The culture of isolated mesothelial cell clones (HBME-1+) show an incredible differentiation capacity in knockout serum cultures with specific growth factors. We were able to specify differentiation to neurons, osteoblats and skeletal muscles (Jahangiri et al, 2007 in press). 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

O-141: Differentiation of Human Embryonic Cells into Osteoblastic Cells

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Human embryonic stem (hESC) cells are derived from inner cell mass (ICM) cells of early mammalian blastocysts. They have the ability to differentiate in vitro and in vivo into derivatives of all three embryonic germ layers (endoderm, mesoderm, and ectoderm). However, there is no current standard protocol for directing differentiation of hESC into mesoderm and osteogenic cells. In my presentation, I will discuss a variety of approaches explored by our lab to direct the differentiation of hESC into osteoblastic cells. I will discuss the following topics: (a) the stage specific expression of several markers of mesoderm lineage during in vitro differentiation of hESC into 3D-structures known as embryoid bodies (hEBs) and the possibility of using these markers to isolate a homogenous population of mesoderm-like cells with osteogenic differentiation potential, (b) protocols directing the differentiation of hESC cultured as EBs into osteoblastic cells. (c) protocols directing the differentiation of hESC cultured as a monolayer into osteoblastic cells. Our data suggest that it is possible to direct the differentiation of hESC into osteoblastic cells based on well-defined in vitro and in vivo criteria. However, the efficiency of differentiation is still not optimal,

posing a major challenge for generating a large number of osteoblastic cells to be employed in clinical applications.

O-142: Human Mesenchymal (Skeletal) Stem Cells: Basic Biology and Clinical Applications for Bone Tissue Regeneration

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Human bone marrow stromal cells (hMSC) contain a population of stem cells called skeletal (mesenchymal) stem cells that are capable for differentiation into several mesodermal-type lineages including osteoblasts, adipocytes and chondrocytes. The aim of our research program is to understand the biological characteristics of hMSC that are relevant for their use in therapy. The following topics will be discussed in my presentation: a) approaches to isolate homogenous population of hMSC from the bone marrow using specific criteria, b) a genetic approach to overcome the limited in vitro proliferative potential and the senescence-associated growth arrest phenotype exhibited by the cells during long-term culture. This approach is based on transducing hMSC with human telomerase reverse transcriptase (hTERT), c) Recent data regarding the molecular control of hMSC differentiation and some new factors that maintain hMSC in undifferentiated state and others that promote their differentiation into osteoblastic phenotype. I will also present our experience with applying state-of-the-art proteomic approaches to studying the biology of hMSC.

O-143: Autologous Cultivated Limbal Stem Cell Transplantation in Cases with Unilateral Limbal Stem Cell Deficiency

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O-144: Differentiation of Human Bone Marrow Derived Mesenchymal Stem Cell (HBMMSCs) to Hepatocyte in PCL Scaffold

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Introduction: At present, bone marrow derived mesenchymal stem cells (MSCs) are considered to have great potential to become one of the best sources for liver tissue engineering. Most studies for hepatic transdifferentiation of adult stem cell have been carried out in monolayer culture. However, hepatocytes are known to better maintain their differentiated functions in three dimensional (3D) matrics. The aim of this study was to investigate the hepatic differentiation of caprolactone) scaffold–human bone marrow derived MSCs (HBMSCs) in Poly
Materials and Methods: In the present study, MSC was isolated from human bone marrow (hBM) by combining gradient density centrifugation with plastic adherence. Cell attachments in scaffolds were examined using scanning electron microscope (SEM). Cultured cell were treated with hepatocyte growth factor (HGF), dexamethasone (DEX), and oncostatin M (OSM) in two step protocol. Differentiating characterization of HBMSCs was detected by immunohistochemistry and ELISA for albumin and alfa-fetoprotein.
Results: SEM micrographs of cells showed that cells adhered and proliferated well on the

outer and inner surfaces of these hybrid scaffolds respectively. The results of immunohistochemistry analysis showed that differentiated cells express albumin and alfa-fetoprotein from day 7 to day 21. The synthesis of albumin in differentiated cells was confirmed by ELISA. Analysis of albumin levels in differentiated cells by ELISA showed a significant increase of albumin expression with time of differentiation.

Conclusion: The presented evidence indicated that HBMSCs differentiate to hepatocyte in PCL scaffold. This scaffold can be implanted into the liver or used in bioartificial liver devices and show promise for future liver tissue engineering.

O-145: Generation and Phenotypic Analysis of *Klf13*$^{-/-}$ Mice

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The Krüppel-like factor *Klf13* is a member of a family of transcription factors several of which are critical for the development of cardiovascular system and the differentiation of haematopoietic cells. *Klf13* is expressed in most murine tissues including heart, vasculature, thymus, bone marrow, and spleen.

Phenotypic changes in a number of cell types have been reported to be in correlation with modulation of *Klf13* expression. For example, in vascular smooth muscle cells (VSMCs) *Klf13* expression correlates with the expression of the smooth muscle-specific gene SM22α both *in vitro* and *in vivo*. In other studies, *Klf13* expression increased markedly during mouse erythroleukemia cell differentiation *in vitro*. In addition, *Klf13* has been shown to activate the expression of RANTES in late activated T cells indicating a role for in lymphocytes.

To investigate the role of *Klf13 in vivo*, mice with disrupted *Klf13* allele were generated. The effect of loss of *Klf13* was analysed in cardiovascular system and also on the differentiation of Erythroid, B cell, T cell and myeloid lineages. Adult *Klf13*$^{-/-}$ mice showed an increased frequency of early onset cardiomyopathy at 6 months, and alterations in the expression of some cardiac genes. The cardiac abnormalities are accompanied by a decrease in the expression of SM22 in VSMCs and an increase of 20 mmHg in systolic blood pressure.

In addition, *Klf13*$^{-/-}$ mice showed mild macrocytic anaemia, and altered numbers of circulating leukocytes due to changes in eythropoiesis and lymphopoiesis. In the spleen and bone marrow, there was an increase in the number of immature erythroblasts. In the thymus, there was an accumulation of $CD4^{+}8^{+}$ thymocytes accompanied by a significant decrease in CD4 single positive (SP) cells resulting in a decreased CD4:8 ratio consistent with a reduction in T cell receptor signal strength. These data show an important role for *Klf13* in the development of cardiovascular system as well as differentiation of multiple haematopoietic lineages.

O-146: Successful Stem Cell Therapy Using Umbilical Cord Blood-Derived Multipotent Stem Cells for Buerger's Disease and Ischemic Limb Disease Animal Model

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Buerger's disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vasoocclusive disease. It is characterized

pathologically as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially the distal extremities (the feet and the hands). There is no curative medication or surgery for this disease. In the present study, we transplanted human leukocyte antigenmatched human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) into four men with Buerger's disease who had already received medical treatment and surgical therapies. After the stem cell transplantation, ischemic rest pain suddenly disappeared from their affected extremities. The necrotic skin lesions were healed within 4 weeks. In the follow-up angiography, digital capillaries were increased in number and size. In addition, vascular resistance in the affected extremities, compared with the preoperative examination, was markedlydecreased due to improvement of the peripheral circulation.
Because an animal model of Buerger's disease is absent and also to understand human results, we transplanted human UCB-derived MSCs to athymic nude mice with hind limb ischemia by femoral artery ligation. Up to 60% of the hind limbs were salvaged in the femoral artery-ligated animals. By in situ hybridization, the human UCB-derived MSCs were detected in the arterial walls of the ischemic hind limb in the treated group. Therefore, it is suggested that human UCBderived MSC transplantation may be a new and useful therapeutic armament for Buerger's disease and similar ischemic diseases.

O-147: BIO, GSK3 Specific Inhibitor, Promote Dopaminergic Differentiation of Unrestricted Somatic Stem Cell

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Introduction: Canonical Wnt signaling plays a crucial role in controlling cell expansion in many types of stem cells. Recent studies, however, demonstrated that Wnt is not only a general stem cell growth factor but can also influence cell lineage decisions in certain stem cell types by promoting specific fates at the expense of others Recently introduced pluripotent stem cell USSC an interesting cellular source to promote brain regeneration.in vivo. Differentiation of these cell to heart bone and liver and neuron has been also investigated. The present study aimed to promote the differentiation of unrestricted somatic stem cells into dopaminergic neuron via the Wnt signaling pathway
Materials and Methods: USSCs were isolated from mononuclear cells of the umbilical cord and recognized by specific markers such as CD146, CD166, CD105, CD133, CD34, vwf. Basic fibroblast growth factor and retinoic acid used as neural differentiation factors and 6-bromoindirubin-3-oxime (BIO) a specific pharmacological inhibitor of GSK3.a component different dopaminergic and neuronal markers considered for dopaminergic neuron differentiation status of cells.
Results: Our results showed that in the presence of both neural differentiation and Wnt inducer factors in culture, more differentiated cells with morphological and molecular characteristics of dopaminergic neurons were seen in comparison with cells in the absence of Wnt inducers. Moreover, there was a significant increase in the expression of β-catenin in these cells compared to that in control, confirming that Wnt pathway is activated in these cells.
Conclusion: From this result and the neuroprotective role of canonical wnt signaling it can be suggested that it may be a key signaling for cell tyherapy of Parkinson disease

O-148: Generation of Feeder-Free and LIF-Independent Bovine ES Cell Lines

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Introduction: Bovine embryonic stem cell (bESC) lines were established from Day 8 IVP embryos, on feeder layers with a complex medium containing serum, growth factors and hLIF. These cell lines were cultured for 18 passages (144 days) and embryoid bodies (EBs) formed from ESC explants taken at Passage 15 and cultured without LIF and growth factors. After 2-3 weeks, EBs attached to the dish and were cultured for an additional 20 days. ES-like cells were present in these cultures after 3 weeks, even though no LIF or growth factors were present. It may be possible to establish a LIF-independent bESC line from these ESC-like cells.
Materials and Methods: Putative ESCs were taken from the attached EBs and cultured with or without LIF. The bESCs were analysed for markers of pluripotency. Gene expression of Oct4 and Rex1 was assessed by RT-PCR, protein localisation of three stage-specific embryonic antigens, SSEA1, SSEA3, SSEA4 and Oct4 was investigated by immuno-histochemistry and alkaline phosphatase (ALP) activity was determined by histochemistry.
Results: The initial bESC lines expressed markers of pluripotency at passage 15 including SSEA3, SSEA4, ALP, Oct4 and Rex1 and had a high nucleus to cytoplasm volume, as demonstrated by DAPI staining. In addition, these cells had a normal karyotype of 60 at passage 13. The cell lines from EBs have been cultured for 36 days and have a morphology characteristic of ES cells. At Day 18 the ESC lines from EBs expressed Oct4 and Rex1.
Conclusion: Although the cell lines established from EB cultures must be characterized further, these results indicate that bESCs can be isolated and maintained without a feeder layer and without LIF.

O-149: Induction of Mixed Chimerism by Embryonic Stem Cells in Mice: Do Infused ES Cells Truly Differentiate?

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Since the report by Krause *et al*. (*Cell* 2001) claiming that hematopoetic stem cells (HSC) can differentiate into a wide range of tissues other than hematopietic lineage, a number of studies have challenged the concept of adult stem cell plasticity. In particular, a number of investigations have concluded that trans-differentiation of lineage-committed adult stem cells is a rare event. For example, HSC appear to acquire other lineage-specific characteristics through fusion with other cells rather than undergoing true trans-differentiation (*i.e*., signal mediated).
Embryonic stem cells (ESC) are unique due to their pluiropotency and low immunogenicity. The high degree of plasticity in ESC makes them capable of developing into any specialized tissue under appropriate conditions. Furthermore, because of being immune-privileged, ESC have a high potential for being used in stem-cell-based therapies in allogeneic recipients even without host pretreatment (*i.e.*, immunosuppression). We have shown that ESC induce mixed chimerism in allogeneic recipients and that they home to different organs including the spleen, thymus, and liver. Understanding the true nature of these cells *in vivo*, however, is a prerequisite for their future application in stem-cell-based therapies.
In order to investigate whether ESC fuse with host cells, we employed a Cre/*loxP* recombination-based method. We show that in addition to differentiation into hematopoetic cells, ESC also fuse with bone marrow derived cells both *in vitro* and *in vivo*, albeit to seemingly a much lower percentage. Fused cells were also detected in peripheral blood of mice successfully transplanted with bone marrow cells. Our observations suggest that fusion occurs under physiological conditions, and this does not require pre-organ damage.

O-150: Stem Cells Biology: PU.1 (Sfpi1), a Pleiotropic Regulator Expressed from the first Embryonic Stages with a Critical Function in Germinal Progenitors

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In the adult mammalian testis, spermatogenic differentiation starts from a minute population of spermatogonial stem cells (SSCs). SSCs are generated after birth from the fetal gonocytes, themselves derived from the primordial germ cells (PGCs), which are specified during the first days after implantation. Transcriptome profiling of purified preparations evidenced the preferential accumulation in SSCs of transcripts of *PU.1* (*Sfpi1*), a regulatory gene previously identified in hematopoietic progenitors. In situ immunolabeling and RNA determination showed a complex pattern of expression in the adult testis, first in SSCs and early spermatogonia followed by de novo expression in pachytene spermatocytes. Spermatogenesis in a null mutant ($PU.1^{G/G}$) was arrested at the prenatal stage, with reduced numbers of gonocytes due to a defect in proliferation already noticeable at embryonic day E12.5. Transcripts of several germinal markers including *Vasa* (*Mvh/*, *Ddx4*), *Oct4*, *Dazl*, *Taf4b*, were detected, while *Stella* (*PGC7*/*Dppa3*) was not. Germ cells of of $PU.1^{G/G}$ newborns testes grafted in nude mice did not initiate the expected post-natal replicative stage, while grafts of their heterozygote littermates underwent complete spermatogenesis. During embryonic development, *PU.1* transcription was initiated as early as the blastocyst stage, with a generalized expression at E6.5 in the embryonic ectoderm. *PU.1* appears therefore to play a determinant role in at least two distinct lineages and, given its wide range of expression, possibly in other stem cells.

O-151: RNA-Mediated Induction of Hereditary Epigenetic Modification

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Paramutation, first observed in maize and subsequently in a variety of plants, is a heritable epigenetic change of the phenotype of a "paramutable" allele, initiated by interaction in heterozygotes with a "paramutagenic" form of the locus. Often referred to as an exception to the law of Mendel, which states that genetic factors segregate unchanged from heterozygotes, paramutation is meiotically stable and inherited in the absence of the inducing allele. The closest observations in an animal species were changes in the DNA methylation profiles directed by the allelic locus in the mouse that we and others described as "transvection" or "paramutation-like" effects. Recently, we have reported a modification in the phenotypic expression of the wild type allele of the Kit receptor gene in the progeny of heterozygotes with a null insertion mutant. In spite of a wild type genomic structure, the modified homozygotes maintain the "White Spotted" phenotype characteristic of *Kit* mutants, in this case a white tip of the tail and white feet. This epigenetic modification is efficiently inherited in the absence of the mutant allele. It was related to a decreased level of Kit mRNA, concomitant with the accumulation of RNA molecules of abnormal sizes. On the other hand, transcription of the locus was upregulated in heterozygotes. Sustained expression at the postmeiotic stages, at which the gene is normally silent, led to the accumulation of RNA in late spermatids and in the spermatozoon. Microinjection into one-cell embryos of RNA from $Kit^{tm1Alf/+}$

heterozygotes, or of Kit specific microRNAs efficiently induced a heritable White-Spotted phenotype. Consistent with converging evidence of a role of RNA in the establishment of epigenetic states and with the detection of RNA in human spermatozoa, our results reveal an unexpected mode of epigenetic inheritance by the zygotic transfer of RNA molecules. Observations were at this stage limited to a fur color marker. One may further consider the hereditary transmission of an epigenetic modification as a possible cause of familial disease predispositions. To extend the concept of epigenetic determination to pathophysiological conditions, we proceeded to the microinjection of several series of miRNAs in early embryos. Interestingly, miR-1, a microRNA expressed in muscle cells with a role in myocyte and cardiomyocyte proliferation induced hereditary heart hypertrophy. In crosses of either male or female paramutants with normal mice, hypertrophic cardiomyopathy (HCM) was inherited over at least three generations. The finding of elevated levels of RNA, including miR-1, in the spermatozoa of the paramutated males supports the hypothesis of RNA-mediated inheritance. The mechanism leading from the alteration in fertilized eggs to the establishment of a stable epigenetic change is still a matter of speculation. As it is, the mouse paramutation may provide a model for human familial diseases, including cardiomyopathies, whose inheritance is not fully explained in Mendelian terms.

O-152: Multilineage Differentiation of Rhesus Monkey Embryonic Stem Cells in a Three-Dimensional Culture System

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Most of the in vitro studies on both human and rhesus monkey ESC differentiation were performed in monolayer cultures, while in vivo this process occurs in the context of three-dimensional tissues. Three-dimensional systems provide an environment for ESCs to form tissues and organs. For example, under experimental conditions in SCID mouse, rhesus monkey ESCs differentiated and formed teratoma with structures of ectodermal origin resembling neural tubes, embryonic ganglia, and brain-like gray matter, as well as of endoderm-derived tissues, including intestinal and ductal epithelium and pancreas. Apparently ESCs migrate within the tissues and interact with the tissues' resident cells as well as with non-cellular elements, in particular extra-cellular matrixes (ECMs), which is vastly different from monolayer cultures. ECMs such as collagens play important roles in cell differentiation by providing biological signals to promote and maintain cell differentiation. Together with growth factors ECMs may create distinct cellular microenvironments or 'niches' that locally regulates ESC proliferation and differentiation.
To investigate differentiation of ESCs and formation of tissue-like structures in the context of in vivo-like three-dimensional system, we developed protocols for ESC differentiation in three-dimensional collagen matrix. Under these conditions ESCs migrate into the collagen matrixes, proliferate, differentiate, and form various tissue-like three-dimensional structures. To mimic in vivo situation further we co-cultured ESCs in collagen matrixes with human dermal fibroblasts or keratinocytes. This protocol facilitates ESCs differentiate into a particular lineage, accompanied by the formation of tissue-like structures. Three-dimensional culture systems are a valuable tool for directing ES cell differentiation and formation of organs and tissue for transplantation.

O-153: Primate Embryonic Stem Cells Create their Own Niche While Differentiating in Three-Dimensional Culture Systems

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Rhesus monkey embryonic stem cells (ESCs) (R366.4), cultured on a three-dimensional (3D) collagen matrix with or without human neonatal foreskin fibroblasts (HPI.1) as feeder cells or embedded in the collagen matrix, formed complex tubular or spherical glandular-like structures and differentiated into phenotypes characteristic of neural, epithelial and endothelial lineages. Here, we analyzed the production of endogenous extracellular matrix (ECM) proteins, cell-cell adhesion molecules, cell surface receptors, lectins and their glycoligands, by differentiating ESCs, forming a microenvironment (niche) able to guide cell behavior. The expression of some of these molecules was modulated by HPI.1 cells while others were unaffected. We hypothesized that soluble factors and the niche were critical in directing growth and/or differentiation of ESCs in a 3D-environment. Creating a proper experimental 3D-microenvironment, further modified by ESCs and modulated by exogenous soluble factors, may become an adequate culture system for developmental biology studies.

O-154: Enriched NCAM Positive Cells form Functional Dopaminergic Neurons in the Rat Model of Parkinson´s Disease

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We describe a method of generating an enriched population of NCAM-positive cells from a human teratocarcinoma cell line (NTera2/D1) and their differentiation into midbrain dopaminergic neurons in the absence of the caudalizing factor retinoic acid (RA). NTera2 cells were induced to form embryoid bodies and then to generate nestin-positive cells on treatment with serum-free defined medium supplemented with neurotrophic factors. We enriched the neuroprogenitor population by magnetic sorting of the nestin-positive cells using the antibody to neural cell adhesion molecule (NCAM). These cells were expanded by exposing them to the signaling molecule sonic hedgehog (SHH) in conjunction with fibroblast growth factor-8 (FGF-8).
The predifferentiated cells when analyzed by RT-PCR showed expression of dopaminergic markers such as Nurr1, Engrailed-1, aromatic amino decarboxylase (AADC), VMAT2, tyrosine hydroxylase (TH), and dopamine transporter (DAT). These cells also stained positively for protein markers such as nestin, NCAM, MAP-2, and TH. We further demonstrated that when transplanted into the brain of Parkinsonian rats, these neuroprogenitor cells did not form tumors but differentiated into dopaminergic neurons, as revealed by TH immunolabeling. The origins of transplanted cells were further confirmed by positive immunolabeling with anti-human nuclei. Our results suggest that enriching the neuroprogenitor population by magnetic sorting prevents tumor formation and is a prerequisite before cell replacement therapy for Parkinson's disease.

O-155: Alpha-Fetoprotein Action on Clonogenic Activity, Self-Renewal and Differentiation of Three Bone Marrow Hematopoietic Stem Cell Subpopulations *Ex Vivo*

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Introduction: Alpha-fetoprotein (AFP) could play a regulatory role in hematopoiesis. However, the evidence of AFP involving in related hematopoietic stem cells (HSCs) biological functions is absence. Investigations were in AFP implication study of clonogenic activity, self-renewal and differentiation of mice bone marrow HSCs subpopulations ex vivo.
Materials and Methods: Clonogenic activity was investigated on HSC-CFU Basic medium. Clone phenotypes were analyzed by immunochemical staining in light microscopy. Self-renewal and differentiation were studied by flow cytometry using anti-CD34-FITC antibody, anti-CD38-PE antibody and line marker fluorescent antibody cocktail.
Results: AFP significantly increases HSCs clonogenic activity. Clones consisted of precursors and lower part of clones represented by primitive polypotent cells (CFU-GEMM and CFU-GM). CD34+CD133+- and CD34+CD135+ HSC cultures possessed macrophage precursors (CFU-M) in highest level and insignificantly erythroid precursors (BFU-E). Dominant forms in CD34+CD117+ culture were BFU-E and CFU-E colonies but CFU-M formed slightly. AFP induced HSCs cultures had granulocyte precursors. Self-renewal analysis indicated significantly CD34+CD38- cells expansion at early period but then their numbers decreased with increasing CD34+CD38+ cell numbers. AFP incubations with CD34+CD133+ and CD34+CD135+ HSC supported CD34+CD38- cell numbers until 22 incubation day. However, CD34+CD38- cells not detected in CD34+CD117+ HSC culture in the latest stages. Differentiation analysis of HSCs demonstrated transformation into both monocytes and erythrocytes. Monocytes dominated in CD34+CD133+- and CD34+CD135+ HSC and erythroid cells prevalent in CD34+CD117+ HSC culture. Other line markers weren't detected. Theses AFP effects were specific because none of albuminoid gene family members effected on such HSCs functions but polyclonal antibody against AFP fully blocked AFP effects.
Conclusion: AFP involves in colony-formation and differentiation of three different HSC subpopulations, and possible it plays a role as HSCs specific clonogenic and differentiation factor in vivo. Furthermore, AFP plays role as self-renewal factor for some HSC subpopulations and takes part in general stem maintaining of HSCs.

O-156: Review of the Methodologies for Differentiation of Embryonic Stem Cells into the Distal Airway Epithelium

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Introduction: Cell therapy is a promising approach for management of distal lung diseases. This approach can be highly facilitated by the in vitro methods that can yield the required number of distal airway epithelial cells on demand. As embryonic stem cells (ESCs) can provide unlimited number of cells and have the potential to differentiate into a variety of phenotypes for cell therapy applications, several methods have been tried to differentiate these cells into the distal airway epithelium. Type II alveolar cells were the primary targets for this series of methodologies but Clara cells were also derived.
Text: Growth factors-mediated differentiation was the first methodology employed. The murine and human ESCs were provided with a medium designed for maintenance of mature distal airway epithelial cells in vitro, which directed the cells into the target phenotypes. To increase the yield of target cells, the protocol was modified by addition of activin A in its early phase. Other methodologies employed were co-culture of murine embryoid bodies with fetal lung mesenchyme, and exposure of ESCs to cytoplasmic extracts of mature type II pneumocytes. All these methods increased the differentiation of ESCs into the target phenotypes. The functionality of ESC-derived

type II cells were confirmed by their differentiation into type I cells. But, all these methods resulted in generation of a mixed population of differentiated cells with low-yield of target cells. In a recent study, it was shown that a highly pure population of hES cell-derived alveolar type II cells can be produced by using a genetically modified human ES cell line followed by antibiotic selection. **Conclusion:** These methodologies show that in vitro engineering of distal lung epithelial cells for therapeutic applications can be achieved by combination of several tec.

O-157: Basic Fibroblast Growth Factor Controls Migration in Human Mesenchymal Stem Cells

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Little is known about the migration of mesenchymal stem cells (MSCs). Some therapeutic approaches had demonstrated that MSCs were able to regenerate injured tissues when applied from different sites of application. This implies that MSCs are not only able to migrate but also that the direction of migration is controlled. Factors that are involved in the control of the migration of MSCs are widely unknown. The migratory ability of isolated MSCs was tested in different conditions. The migratory capability was examined using Boyden chamber assay in the presence or absence of basic fibroblast growth factor (bFGF), erythropoietin, interleukin-6, stromal cell-derived factor-beta, and vascular endothelial growth factor. bFGF in particular was able to increase the migratory activity of MSCs through activation of the Akt/protein kinase B (PKB) pathway. The results were supported by analyzing the orientation of the cytoskeleton. In the presence of a bFGF gradient, the actin filaments developed a parallelized pattern that was strongly related to the gradient. Surprisingly, the influence of bFGF was not only an attraction but also routing of MSCs. The bFGF gradient experiment showed that low concentrations of bFGF lead to an attraction of the cells, whereas higher concentrations resulted in repulsion. This ambivalent effect of bFGF provides the possibility to a purposeful routing of MSCs.

O-158: Genomics of Endometrial Receptivity

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The diagnostic of endometrial receptivity based on endometrial dating has been used worldwide for the last 50 years. Histological interpretation is inherently subjective, and intraobserver variability has been shown to be highest among infertile women during the implantation window. New molecular tools allows us to recognize the gene expression pattern of the human endometrium at the time of implantation by comparing receptive versus non-receptive endometrium or receptive versus pre-receptive. In our laboratory, we have investigated the dynamics of the gene expression profile in the human endometrium throughout the window of implantation (WOI) as an attempt to dissect out molecular markers of endometrial receptivity. We have collected endometrial samples (n=25) from healthy fertile normal cycling women (aged 23-39), at days LH+1, LH+3, LH+5, LH +7 and LH+9 of their natural cycles (n=5 each time point) by using a Pipelle catheter (Genetics, Namont-Achel, Belgium) under sterile conditions from the uterine fundus. We have performed histological studies for endometrial dating and microarray analysis for determining the gene expression profile throughout that period of time. We have analyzed the expression data by using different methods

such as clustering or selection of differentially expressed genes, as implemented in the GEPAS.
Gene Ontology analysis demonstrated that the functional categories that were overexpressed at the different days throughout the WOI were different. At the day of blastocyst adhesion, the gene expression profile in the receptive endometrium at LH+7 shows stricking differences. We have identified a cluster of 60 genes that specifically are up-regulated during the WOI at LH+7 and remained up-regulated at LH+9 presenting a specific receptive profile. These data have been compared and validated with those obtained in previous studies.
Our work in the genomics of endometrial receptivity indicates that the endometrial gene expression pattern undergoes unequivocal changes throughout the WOI with a defined gene expression profile. Endometrial genomic profile and functional annotation during the WOI under proper bioinformatics analysis offers the possibility to be an objective predictor of endometrial receptivity.

O-159: Stem Cells in Reproductive Medicine

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The stem cell field owes much to previous work developed by embryologists and researchers devoted to Reproductive Medicine. The time is coming and this emerging field is paying off to Reproductive Sciences offering new avenues of understanding gametogenesis and early embryonic development. In mammals, primordial germ cells set aside during early embryonic development give rise to the production of male and female gametes. Once the sperm enters the egg, the two pronuclei become enveloped, fuse and begin the zygote´s mitotic cycle. The earliest developmental events are regulated by maternally inherited mRNA whereas genomic activation occurs at 6 to 8-cell stage in humans. From the preimplantation embryo, at morula or blastocyst stage or even from totipotent blastomeres derivation of embryonic stem cells can be achieved. The possibility to accomplish the differentiation of gametes from embryonic stem cells or/and from progenitor stem cells present in the gonads or other organs will complete this enigmatic “Circle of Life”.
Embryonic stem cells proliferate in vitro while maintaining an undifferentiated state, and are capable of differentiating into most cell types in the appropriate conditions. These properties imply great potential in the treatment of various diseases and disabilities. However, the therapeutic application for human embryonic stem cell derivatives is compromised by the exposure of existing lines to animal and human components, with the subsequent risk of contamination by retroviruses and other pathogens, which can be transmitted to patients (Gearhart, 2004).
Initial derivations of hESCs were performed in the Thomson´s laboratory using the ICMs of human blastocysts placed on inactivated murine feeder cells (MEF). More than seventy-eight hESC lines are available on the NIH registry, but all have been derived and propagated on MEF (xenosupports) in the presence of animal-based proteins (xenoproteins). The use of feeders from mice and products from animal sources raises the possibility of zoonosis. Contamination by animal products represented less of a theoretical concern when it was found that hESCs cultured with animal cells or serum products could take up Neu5Gc, a non-human sialic acid that is immunogenic in cells used for human transplantation.
The main objective at present, is to determine the minimal combination of growth factors that maintain the proliferation and pluripotency of undifferentiated hESCs without the need for feeder cells or conditioned medium. In this context, three new types of medium that minimize animal exposure and improve culture definition have evolved. A recent report presented a feeder-independent method that permits hESC culture and novel derivations have been produced including protein components derived solely from recombinant sources from human materials.
According to new EU directives (2003/94/EC and 2004/24/EC), human embryonic stem cells for transplantation must be cultured using conditions of Good Manufacturing Practice (GMP), in order to guarantee the

safety and quality of the cells. Incorporating GMP conditions implies impeccable record keeping, qualified personnel, high sanitary standards, equipment verification, validation of processes and complaint management. In addition, conditions must be defined and xeno-free so that the risk of zoonosis is eliminated, and feeder-free systems need to allow large scale production.
Embryo-friendly approaches are currently being developed as new methods of obtaining hESC without destroying the embryo. The most attractive approach being by removing a reduced number of blastomeres, maintaining the viability of the embryo while the material extracted can be used to obtain hESC. The blastomeres removed, could be used for hESC derivation, and it has been already proven feasible in the mouse model. This technical approach would offer a source of hESC without causing the destruction of the human embryo and, in the case of embryos with a predisposition to hereditary diseases, would offer the possibility of establishing a hESC bank for future regenerative medicine.
In this presentation, we summarize the attempts that have been made to prepare hESC for Regenerative Medicine.

O-160: Pharmacoproteomics: Strategies for Identifying New Diagnostic and Prognostic Markers for Colorectal Cancer

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Colorectal cancer (CRC) is a leading cause of cancer death in the Western World. Early detection is the single most important factor influencing outcome of CRC patients. If identified while the disease is still localized CRC is treatable. To improve outcomes for CRC patients there is a pressing need to identify biomarkers for the early detection (diagnostic markers), prognosis (prognostic indicators), tumor responses (predictive markers) and disease recurrence (monitoring markers). Despite recent advances in the use of genomic analysis for risk assessment, in the area of biomarker identification genomic methods have yet to produce reliable candidate markers for CRC. For this reason, attention is now being directed towards protein chemistry or proteomics as an analytical tool for biomarker identification. Here, we discuss various proteomics technologies with reference to how they may contribute to CRC biomarker discovery. One such strategy uses a combination of continuous free flow electrophoresis (FFE) in the first dimension, a liquid-based IEF technique, followed by rapid RP-HPLC (1-6 min/analysis) in the second dimension. Imaging software has been developed to present the FFE/RP-HPLC data in a virtual 2D format. Demonstration of the method is presented using proteome analysis of human plasma and urine specimens. Additionally, we describe strategies for analyzing the 'secretome' of human CRC cell lines, the human platelet membrane proteome, tissue interstitial fluid from tumor-derived human colon carcinoma cell line xenografts, and a proteomics approach for analyzing the effect of non-steroid anti-inflammatory drugs on human colorectal carcinoma cell lines.

O-161: Cell-Based Therapies for Treatmemt of Ischemic Heart Disease: Fact or Fiction?

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O-162: Umbilical Cord-Derived Cell Products for Treatment of Congenital Heart Disease

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O-163: Developmental Plasticity of Luteinizing Granulosa Cells Cultured over Prolonged Time Periods in Culture Medium Supplemented with the Leukemia-Inhibiting Factor

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The mature follicle, at preovulatory stage, consists of follicular fluid, one single mature oocyte, surrounded by several hundred thousands of granulosa cells (GCs). Luteinizing GCs were isolated from the ovarian follicular aspirates of patients treated with assisted reproduction and isolated and sorted with flow cytometry based upon the presence of the FSH receptor (FSHR). These cells, considered as terminally differentiated, could be maintained over prolonged periods of time when cultured in the presence of the leukemia-inhibiting factor (LIF). During prolonged culture the markers of GC function such as FSHR and aromatase slowly disappeared. OCT4, a typical stem cell marker, was expressed by GCs, but not germ line cell markers such as nanog, vasa and stellar. Mesenchymal stem cell (MSC) markers such as CD29, CD44, CD90, CD105, CD117 and CD166, but not CD73, were expressed by subpopulations of GCs. In order to further establish the MSC nature of these cells, they were transferred into specific culture media, where they differentiate towards other cell types, otherwise not present within ovarian follicles, such as neuronal cells, cartilage and bone.

O-164: NPC1 Gene Deficiency Leads to Lack of Neural Stem Cell Self-Renewal and Abnormal Differentiation through Activation of p38 Mitogen-Activated Protein Kinase Signaling

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Neural stem cells (NSC) are capable of giving rise to neurons, glia- and astrocytes. Although self renewal and differentiation in neural stem cells are regulated by many genes such as Notch, Numb etc., little is known of the role of defective genes on the self renewal and differentiation of neural stem cell from developing brain. The Niemann-Pick type C1 (NPC1) disease is one of the neurodegenerative diseases, caused by a mutation of NPC1 gene which affects the function of NPC1 protein. The ability of NSC self renewal and differentiation was investigated using a model of Niemann-Pick type C1 (NPC1) disease. The NPC1 disorder significantly affected the self renewal ability of neural stem cells, as well as the differentiation. Neural stem cell from *NPC1*$^{-/-}$ mice showed impaired self renewal ability when compared to the *NPC1*$^{+/+}$ mice. These alterations were accompanied by the enhanced activity of p38 MAP kinases. Further, the specific p38 MAP kinase inhibitor, SB202190 improved the self renewal ability of NSCs from *NPC* $^{-/-}$ mice. This indicated that the NPC1 deficiency can lead to lack of self renewal and altered differentiation of neural stem cells mediated by the activation of p38 MAP kinase impairing the generation of neurospheres from *NPC1*$^{-/-}$. Thus the NPC1 gene may play a crucial role in NSC self-renewal associated with p38 MAP kinase.

O-165: Microgravity Potentiates Stem Cell Proliferation While Sustaining the Capability of Differentiation

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Introduction: Bone marrow-derived human mesenchymal stem cells (hMSCs) have received much attention in recent years due to their potential in the field of regenerative medicine. However, the low proliferative ability and limited life span of hMSCs has thus far restricted their wider application.
Materials and Methods: A three-dimensional (3D) clinostat is a recently developed device for generating multi-directional G force, resulting in an environment with an average of 10-3 G. We cultured hMSCs in a 3D-clinostat (group CL) and compared them with hMSCs cultured in a normal 1G environment (group C). We then transplanted a pellet of hMSCs cultured under each culture condition for 7 days into cartilage-defect mouse models.
Results and Conclusion: The group CL cells showed marked proliferation (13-fold in a week) compared with group C cells (4-fold in a week). Flow cytometry revealed a 6-fold increase in the number of hMSCs double-positive for CD 44/CD 29 or CD 90/CD 29 in group CL after 7 days in culture, compared with group C. Telomere length remained the same in cells from both groups during culturing. The group C cells showed increasing expression levels of type II collagen and aggrecan over the culture period, while group CL cells showed a decrease to undetectable levels. Pellets of hMSCs from each group were explanted into cartilage-defect mice. The transplants from group CL formed hyaline cartilage after 7 days, whereas the transplants from group C formed only non-cartilage tissue containing a small number of cells. These results show that hMSCs cultured in a 3D-clinostat possess the strong proliferative characteristic of stem cells and retain their ability to differentiate into hyaline cartilage after transplantation. On the contrary, cells cultured in a 1-G environment do not maintain these features. Simulated microgravity may thus provide an environment to successfully expand stem cell populations in vitro without culture supplements that can adversely affect stem cell-derived transplantations. This method has significant potential for regenerative medicine and developmental biology.

O-166: A Unique Human Blood-Derived Cell Population Displays Embryonic Markers and High Potential for Producing Insulin

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Blood can provide a valuable source for the generation of stem cells. Herein we identified a novel cell population from adult human blood, designated peripheral blood insulin-producing cells (PB-IPC). Phenotypic analysis demonstrated that PB-IPC displayed the embryonic stem (ES) cell-associated transcription factors including Oct-4 and Nanog, along with the hematopoietic markers CD9, CD45, and CD117; but lacked expression of the hematopoietic stem cell marker CD34 as well as lymphocyte and monocyte/macrophage markers. Notably, in *vitro* and in *vivo* characterization revealed that PB-IPC demonstrated characteristics of islet β cell progenitors including the expression of β cell-specific insulin gene transcription factors and prohormone convertases, production of insulin, formation of insulin granules, and the ability to educe hyperglycemia and migrate into pancreatic islets after transplantation into the diabetic mice. These findings may open up new avenues for autologous blood stem cell-based therapies for diabetes.

Poster Presentation

Andrology

P-1: The Effects of Nitric Oxide Synthase Inhibitor (L-NAME) on Epididymal Sperm Count and Morphology in Varicocelized Rat

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Introduction: Nitric oxide has been reported to be increased in the spermatic veins of men affected by varicocele that has a role in testicular dysfunction. L-NAME is a Nitric Oxide Synthase inhibitor. Although Several authors have considered the relationship between varicocele and semen NO concentrations, but no one considered about the Effects Of Nitric Oxide Synthase Inhibitor (L-NAME) On Epididymal Sperm Count And Morphology. The concentration and morphology of spermatozoa are also important as these have correlation with fertility of the individual. Some authors have stated that a partial obstruction of the spermatic vein is the only procedure able to induce a varicocele closest to that of human being.
Materials and Methods: twenty four Wistar male rats divided into three groups. In the group A and B, underwent a left experimental varicocele (partial spermatic vein ligation). By this method the lumen of the vein was reduced to 20-gauge effectively. Group C (control group): These rats underwent a similar procedure without ligation of the spermatic vein.
Animals in group A were killed 10 weeks after the operation and both left and right Epididymal sperm count and morphology were analyzed. Animals in group B were receiving 10mg/kg L-NAME daily intraperitoneal for 10 weeks. After 10 weeks both the left and right epididymal sperm count and morphology were analyzed.
Results: Both Sperm count and morphology was significantly decreased in left epididymis in group A (non treated). There were statistically significant differences between the groups A and B (treated) in production and sperm morphology ($p<0.05$). There were statistically significant differences between the left and right sperm count in the groups A and B ($p<0.05$). There were not statistically significant differences between the left and right sperm morphology in the groups A and B ($p<0.05$).
Conclusion: These findings suggest that nitric oxide synthase inhibitor (L-NAME) improves sperm count and morphology that are associated with infertility in varicocelized rat.

P-2: *In Vitro* Effect of Clomiphene Citrate on Motility and Vitality of Sperm in Mouse

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Introduction: Various approaches have been used in attempts to improve the in vitro quality of sperm, including addition of various additives and drags. Clomiphene citrate (Clomid) is one of the drugs that was used in the treatment of male infertility and has been added for improvement of in vitro sperm parameters. For evaluation of Clomiphene citrate supplementation on sperm characteristics, we investigated the effect of different doses of Clomiphene citrate on mouse sperm.
Materials and Methods: For this purpose we removed cauda epididimes from male mice with aseptic method. Then were supplemented 0.001, 0.01, 0.1, 1 and 10 µg/ml Clomiphene citrate to sperm. Motility parameter was measured under inverted microscope by observation and to analysis sperm viability Eosin Nigrosin staining was used.
Results: Clomiphene citrate supplementation (0.1 µg/ml) improved motility parameters ($p<0.05$) and increased the vitality of sperm ($p<0.05$). The effect of doses of 0.01 and 1 µg/ml also were positive on them. But doses 0.001 and 10 µg/ml had no significant effect on mentioned characteristic.

Conclusion: The dose of 0.1 µg/ml of Clomiphene citrate had the greatest positive effect on the motility and vitality of in vitro sperm.

P-3: Varicocele and Antisperm Antibody: Fact or Fiction?

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Objective: To study the level of antisperm antibodies (ASAB) in serum and semen of infertile men with varicocele, before and six months after varicocelectomy.
Materials and Methods: We performed a prospective study of 81 infertile men undergoing microsurgical inguinal varicocelectomy. Female factor was excluded. Semen analysis, seminal and serum ASAB (direct, indirect IgG and IgA) as measured by sMar test were performed pre and postoperatively at 6 months. A control group including idiopathic infertile patients was evaluated for ASAB.
Results: 81 patients with mean age of 28.7 years (23-42) completed our study. Mean sperm count, motility and abnormal forms were improved postoperatively, significant statistically for sperm density and morphology ($p<0.05$). Preoperatively, twenty-one patients (26%) were low probable positive for ASAB (10-40%), out of which ASAB titer reduced in 15 (A group), increased in 3 (B group), reduced in that particular antibody type but increased in another in 3 (C group) six months after varicocelectomy. In A group sperm count, motility and normal forms improved postoperatively ($p<0.05$). In B group motility reduced postoperatively. In C group motility and normal forms reduced postoperatively. Sixty patients were negative for ASAB preoperatively. Out of these, 48 showed an increase in at least one of ASAB types to some degree that had no significant effect on semen parameters. In control group, 2 patients (7%) were weak positive for ASAB.
Conclusion: Varicocelectomy may reduce ASAB level. This reduction has good effect on semen parameters quality. Also, it may arise ASAB level in some patients. This positive conversion has no adverse effect on semen parameters.

P-4: Is Seminal Plasma Fructose Concentration Associated with Determinants of Semen Quality?

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Background: Fructose is produced in humans mainly by the seminal vesicles with a small contribution from the ampulla of the ductus deferens and is essential for spermatozoa metabolism and motility as energy source.
Materials and Methods: To investigate the correlations between seminal plasma fructose concentration and semen parameters, 69 fertile and 251 infertile males provided a standardized semen specimen. Fructose concentration was determined by acid resorcinol calorimetric method. Semen analysis was performed according to World Health Organization guidelines.
Results: The fructose concentration, agglutination, vitality, sperm count, total motility, rapid motile, none-motile, normal morphology, amorphous head, vacuolated head, small acrosomal area, tail defects, short tail, coiled tail, two tail, three tail, germinal cell and RBC were significantly different between infertile and fertile males. The strong correlations were found between fructose concentration and liquefaction, agglutination, vitality, sperm count, total motile, rapid motile, slow motile, flagella, none-motile, normal morphology, macro head, neck defects, bent neck, tail defects, coiled tail in infertile group.

Conclusion: In conclusion, the present results indicated that seminal plasma fructose concentration can be a good marker for determination of semen parameters especially motility, morphology, vitality and sperm count.

P-5: Correlation between Seminal Plasma Glutathione Peroxidase Enzyme Activity and Semen Parameters

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In the present study, correlations between seminal plasma glutathione peroxidase enzyme activity and semen parameters are evaluated in 240 males. Semen analysis was performed according to World Health Organization guidelines. The 240 males were subdivided into 5 groups as normospermia, oligozospermia, asthenozospermia, azospermia and varicocele according to their spermiogrames. Seminal plasma glutathione peroxidase enzyme activity was determined by Kit (Randox, Germany). The result showed that glutathione peroxidase enzyme activity is higher in normospermic than oligozospermia, asthenozospermia, azospermia and varicocele groups. Also, there are significant and negative correlations between glutathione peroxidase enzyme activity and seminal plasma fructose concentration, white blood cell, tail defects of sperm, coiled tail sperms and short tail sperms. On the other hand, the present data showed that significant and positive correlations between vitality, sperm count, motility and normal morphology. So, the present study showed that measurement of glutathione peroxidase enzyme activity could be a good marker for evaluation of male infertility.

P-6: Hormonal Abnormalities in Azoospermic Men in Kano, Northern Nigeria

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Background and Objectives: We undertook this study to observe the pattern of hormonal abnormalties and testicullar pathology in azoospermic male africans in Kano, northern Nigeria
Materials and Methods: Eighty consecutive azoospermic infertille males attending fertility clinic in Aminu Kano Teaching Hospital, Kano were selected for the study. Their semen were analysed three times at eight weeks interval, after which serum follicle stimulating hormone (FSH) luteining hormone (LH), testosterone and prolactin were assayed in serum samples and histological examination of testicular biopsies done.
Results: Of the 80 subjects studied, 32(40%) had abnormall hormonal levels, 48(60%) had normal hormonal values and 36(45%) had testicular pathology.
Interpretation and Conclussion: Endocrinopathies are common in azoospermia. Their contribution to male factor infertility cannot be overemphasied. The main reason for the endocrinopathies is not known but enviromental factors,, endocrine disruptors and genetic polymorphism have been suggested to be contributory.

P-7: Effect of Alpha-Tocopherol Supplementation on Human Semen Quality and Sperm Parameters

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Introduction: Since antioxidant reduces oxidative stress and improves sperm motility, it could be of clinical significance in the management of male infertility. This study was aimed to find out the efficacy of alpha-tocopherol (vitamin E) in reversing the free radical mediated oxidative damage on sperm motility and morphology.
Materials and Methods: Human semen samples were obtained from the Vali-e-asr Hospital. Each human ejaculation was spilt in to two equal fractions. The spilt seminal fractions were centrifuged at 300 g; 10 min and supernatant was discarded. In the experimental group 2 m M vitamin E was added to medium and after one hour the parameters of sperm were analyzed according to WHO criteria. S.E.M.±Data were analyzed by T-test and All values were given as means Statistical significance was indicated by a P value less than 0.05.
Results: There was a significant increase in the sperm motility rate in group which supplemented with vitamin E ($p<0.05$). Progressive motility was significantly increased in the experimental group.
Conclusion: Supplementation of preparation media with alpha-tocopherol has benefits for sperm motility rate and may be of clinical value in assisted conception procedures.

P-8: Assessment of Cytotoxic Effects of I.V. Injection of Omnipaque 350 on Spermatogenesis in Mouse Model

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Introduction: The worldwide use of non ionic contrast media is being increased very fast. There are not many studies on effects of these materials on spermatogenesis. This research was performed to study the probable cytotoxic effects of omnipaque 350 (a non ionic iodinated contrast material) on spermatogenesis in animal model.
Materials and Methods: The contrast material was injected (2.5 ml/kg) to 5 groups of 3 male Swiss albino mice. Each group of mice was killed in the 18, 20, 22, 24, and 26 days after injection respectively and 20 days after injection was found the time that the number of sperm cells decreases the least. Then different doses of 0.5, 1, 2, 3, 4 ml/kg of omnipaque 350 were injected via tail vein of the 5 groups of 5 mice. The mice were killed 20 days after injection. Testis weight of all groups of mice was compared with the control group and then their testicular sperm heads were counted using haemocytometer under a light microscope (×400). The mean numbers of sperm heads in each group were compared with the control group and survival fractions (SF) were measured.
Results: The mean numbers of sperm heads in all injected groups in comparison with control group showed statistically significant differences in all doses of omnipaque 350. The most decrease in the number of sperm heads occurred at lowest dose (1.0 ml/kg) ($p<0.001$). Testis weight loss was also noted within the same group in comparison with the control group ($p=0.057$). The figure clearly shows the decrease of survival fractions of sperm heads in all doses of omnipaque 350 on spermatogenesis.

P-9: Semen Changes after Extracorporeal Shockwave Lithotripsy for Distal Ureteral Stones

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Objective: To determine the effects on semen of extracorporeal shockwave lithotripsy for distal-ureteral stones.
Patients and Methods: We examined the semen of 62 patients 1 day before and 1 week and 3 months after SWL for distal-ureteral stones. The results were compared with those of 62 patients similarly treated for upper-ureteral stones.
Results: Sperm density and motility decreased significantly after SWL for distal-ureteral stones (*P*-0.001 for both). Macroscopic hemospermia was reported by 16 patients. These changes were reversed by 3 months. No change was observed after SWL for upper-ureteral stones.
Conclusion: Transient deterioration of semen characteristics can be expected after SWL for distal-ureteral stones, which may be attributable to damage to the seminal vesicles or ejaculatory ducts. The changes are transient, and normal semen characteristics are found by 3 months after SWL. The long-term effects in subfertile men warrant further investigation.

Embryology

P-10: *In Vitro* Meiotic Maturation of Mouse Oocytes: Role of Nitric Oxide

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Introduction: In this experiment we used cultured mouse cumulus cell-enclosed oocytes (CEOs) and denuded oocytes (DOs) to study the function of nitric oxide (NO) in mouse oocyte meiotic maturation.
Materials and Methods: CEOs or DOs were cultured for 24 h in a medium containing 4 mM hypoxanthine (HX) to maintain meiotic arrest, or in maturation medium (without HX) supplemented with different doses of sodium nitroprusside (SNP, a NO donor), N-omega-nitro-L-arginine methyl ester (L-NAME) (inhibitor of NO synthase).
Results and Conclusion: NOS inhibitor suppressed the formation of first polar body (PB1) of the oocytes in CEOs in a dose dependent manner, but no effect on germinal vesicle break down (GVBD) was observed. An optimal inhibitory effect was observed with 10–3 M, L-NAME ($p<0.0001$) and the inhibition could be reversed by the addition of SNP (10-5 M). The above mentioned optimal concentration of L-NAME on CEOs exhibited no effect on oocyte meiotic maturation of DOs. Treatments of low concentrations of SNP (10-6, 10-5M) stimulated significantly the oocyte meiotic maturation of CEOs which were inhibited with HX, but had no effect on DOs in the same culture medium. While, the treatment with high concentrations of SNP (0.1-2mM) during the CEOs cultured in maturation medium resulted in a lower percentage of oocytes at PB1 stage and a higher percentage of atypical oocytes in a dose dependent manner compared with control. A dose of SNP at 1 mM exhibited significant inhibitory effect on the formation of PB1, and the number of atypical oocytes compared with control. Oocytes of all groups underwent GVBD at the end of the culture in the spontaneous maturation medium; the results showed that the treatment with the 1mM concentration of SNP could significantly delay GVBD during the first 5 h culture period. The concomitant addition of L-NAME with SNP did not reverse the inhibitory effect of SNP on CEOs. Pre-incubation used of SNP did not have any effect on oocyte maturation. These data support the idea that NO could act in mouse meiotic maturation depending on its concentration.

P-11: Effect of Different Concentrations of Bovine Preovulatory Follicular Fluid and Bovine Fetal Cord Serum on the Development of Two-Cell Mouse Embryos *In Vitro*

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Objective: To measure fetal developmental potential of mouse two-cell cleaved in modified Ham's F-10 medium (Sigma Chemical Co., St. Louis, MO) containing the bovine preovulatory follicular fluid(BFF), bovine fetal cord serum (BCS) and bovine serum albomine(BSA)as a model for establishing criteria for human IVF and GIFT procedures.
Design: Optimum concentrations of CFF,CCS in modified Ham's F-10 were established by measuring blastocyst development of in vivo fertilized zygotes from a mouse strain. **Animals:** eight-week-old, superovulated mice. **Result:** In vivo-derived embryos were cultured in Ham's F-10, to which one of the following substances was added: [1] BSA (4 mg/ml), [2] different concentrations (10 and 20%) BFF, [3] different concentrations (10 and 20%) of BCS added two-cell stage. The proportion of embryos developing was affected by the type of protein and concentration. Reduced cleaved rates of embryo development were observed in Ham's F-10 supplemented with 10%BFF, 10%BCS. The rates of development of balstocystes in vitro were suppressed when the embryos were cultured with 10%BFF or10%BCS as compared with BSA. Ham's F-10 supplemented with 20%BCS, 20%BFF and BSA also supported the development of in vitro-derived embryos ($p<0.05$).
Conclusion: These results suggest that the higher concentrations of protein supplements are more effective for embryo culture.

P-12: Evaluation of the Spindle Apparatus of *In Vitro* Matured Mice Germinal Vesicle Oocytes After Vitrification

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Introduction: Routine oocytes cryopreservation remained an elusive technique in the wide range of assisted reproductive technologies available. The microtubules of oocytes are vulnerable to cryoprotectants and thermal change. This study examines the effect of a vitrification protocol on the spindle and chromosome configurations of mice oocytes cryopreserved at the germinal vesicle stage.
Materials and Methods: Germinal vesicle with cumulus cells were transferred to vitrification solution which was composed of 30% (v/v) ethylene glycol, 18% Ficoll-70 and 0.3 M Sucrose either by single step or in step-wise way. After vitrification and storage in liquid nitrogen, the oocytes were thawed and washed tow time in medium TCM199 composed 20% FBS. Following vitrification and in vitro maturation (MII), the matured oocytes were immonostained for meiotic spindles and chromosomes, before visualization using fluorescent microscopy. **Results:** A statistically significant increase was observed in the survival and maturation rate in step-wise vitrification (88.96%, 71.23% respectively) compared to single step vitrification (70.6%, 62.42% respectively) ($p<0.05$). Normal spindle morphology after vitrification-thawing in step-wise vitrification group (77.26%) was higher than single step vitrification group (64.24%) but lower than control group (94.75%) ($p<0.05$).
Conclusion: The results suggest that mice germinal vesicle oocytes vitrified with step-wise procedure had positive effective on survival and maturation rate and normal spindle configuration than single step vitrification procedure.

P-13: Inhibitory Effects of Müllerian Inhibitory Substance (AMH) on FSH-Induced *In Vitro*

and *In Vivo* Growth and Development of Mouse Follicles

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Objectives: Ovarian follicle growth is under the influence of FSH, one of the most prominent regulators. Therefore, factors affecting the sensitivity of ovarian follicles to FSH are also important for follicle growth. The aim of the present study was to investigate whether anti-Müllerian hormone (AMH) has an inhibitory effect on follicle growth by decreasing the sensitivity of ovarian follicles to FSH. Furthermore, the combined action of AMH and FSH on ovarian follicle development was examined.
Materials and Methods: Preantral follicles (90–100 µm in diameter) were harvested from 6-8 week-old Syrian mice and cultured in TCM199 culture medium for 6 days to see the effect of FSH and recombinant rat anti-Müllerian hormone (MIS). Concentrations of anti-Müllerian hormone were in the range of 100-1000 IU/ml, while 100 mIU/ml FSH was used in the experiment. Three different experiments were performed.
Results: Using an in vitro follicle culture system, it was shown that FSH stimulated preantral follicle growth (300 µm diameter, $p<0.05$) during 6-days experiment and follicle diameter was checked after every second day. In second experiment, it was seen that the growth was attenuated (240 µm diameter, $p<0.05$) in the presence of AMH. In a third experiment, an in vivo examination of the follicle population of 6-week-old wild type, FSH-, AMH- and AMH-/FSH-deficient females revealed that loss of FSH expression has no impact on the number of primordial and preantral follicles but the loss of inhibitory action of AMH on the recruitment of primordial follicles in AMH-deficient mice is increased in the absence of FSH.
Conclusion: In conclusion, these studies show that AMH inhibits FSH-stimulated follicle growth in the mouse, suggesting that AMH is one of the factors determining the sensitivity of ovarian follicles for FSH and that AMH is a dominant regulator of early follicle growth.

P-14: Vitrification of Human Oocyte Using Cryoloop

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Introduction: The cryopreservation of human oocyte would make a significant contribution to infertility treatment, such as using it for oocyte donation and for patients a bout to lose ovarian function due to surgery or chemotherapy. Despite of using standard freezing straws and cryovials or even open pulled straws, only a few successful pregnancies have been arisen from cryopreserved human oocytes. This situation has been primarily attributed to poor survival, fertilization and development of cryopreserved oocytes.
Objective: The aim of this study was to evaluate the novel cryoloop vitrification method for cryopreservation of human oocytes.
Materials and Methods: Nine infertile couples participated in this study. In all women proper regulation and desensitization was done using GnRH agonist during luteal phase. Mature oocytes allocated into two groups randomly. In group I, 34 oocytes were vitrified in conventional straws, while in group II, 33 oocytes were vitrified in cryoloop. After a store time of 1-6 months the oocytes were thawed, incubated for 2 hours and subsequently the ICSI was done on survived oocytes. To verify normal fertilization of vitrified oocytes the number of pronuclei in the cytoplasmwas counted 16-18 hours after ICSI and good morphological quality embryos were transferred on day 2 or 3 after sperm injection. Pregnancy was identified by the serum ß HCG level, checked 14 days after embryo transfer.

Results: The present study shows that the rate of survival of vitrified human oocytes in two groups has no significant difference (52.94% in group I versus 63.63% in group II) but the fertilization rate of vitrified oocytes by cryoloop was greater than vitrified oocytes by conventional straws (73.7% versus 55.55% respectively). One of the embryo transfers achieved clinical pregnancy and resulted in the delivery of healthy baby.
Conclusion: Vitrification by using cryoloop can improved the fertilization rate and developmental capacity of vitrified thawed oocyte

P-15: Affirmation of Embryo Transfer at Blastocyst Stage in Assisted Reproductive System

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Introduction: At recent years, extended embryo culture conditions, better understanding of pre implantation physiology together with amelioration of embryo selection methods, have allowed a substantial increase on both implantation rate and pregnancy in ART. Surely choose of embryo stage in these cycles is one of the important factors in improvement of implantation rate, decrease of the rate of twain pregnancy, successfully in pre implantation genetically diagnosis. Numerous studies were accomplished in the field of embryo transfer in variant stages of embryo transfer. Present study suggested that Blastocyst transfers give significantly higher chance of pregnancy and implantation rate per cycle.
Materials and Methods: NMRI female mice were stimulated with hMG and hCG for super ovulation, and were mated with the same strain males. 150 obtained blastocysts were cultured in Matrigel+M16 medium that supplemented with 4mg/ml BSA (72h). Developmental processes were studied every 24 hours until 72 hors.
Result and Discussion: Morphological studies of developmental statue were demonstrated that embryo has high vital ability and high affinity in attachment to extra cellular matrix. So preservation of embryos until blastocystic stage can affect on improvement of ART results.

Epidemiology and Ethics

P-16: Attitudes toward Menopause, Quality of Life, and Decreased Sleep Quality among Middle-Aged Women: A Community Survey in an Island of Taiwan

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The purpose of this report is to explore the attitudes toward menopause, to investigate the quality of life, and to assess any sleep quality. In addition, related factors about those issues were examined.
A large community-based sample of Taiwanese women aged 40–54 years who are living on the islet of Kinmen were recruited for this study. Menopausal status was determined from menstrual patterns. Attitudes toward menopause were collected with a self-administered questionnaire. Quality of life was assessed by a 2-year longitudinal survey of SF-36. Sleep quality of measured by self-reported sleep problems.
Conclusively, women in Taiwan held a more positive attitude toward menopause as compared to prior studies in other countries. They had a more positive attitude when they actually faced menopause. Education and vasomotor symptoms had a significantly negative impact on menopausal perceptions.

There was no significant effect of menopausal transition on quality of life among Taiwanese women. The decline in the role limitations due to emotional problems was related to vasomotor symptoms. In terms of sleep quality, half of middle-aged Taiwanese women felt dissatisfied with their sleep. Sleep trouble was most frequent in post-menopausal women, followed by peri-menopausal and pre-menopausal status. Anxiety had a crucial factor for sleep quality.
This report provides more insight on the perception of menopausal life in Taiwanese women that will guide future public health initiatives.

P-17: Bioethics in Maternity Research

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When a woman is pregnant, in labor, or breastfeeding agrees to take part in research, she is consenting for two people, herself and her child there for special consideration, should be given by researchers and ethics committees to research on this group. According to pregnant women research protocol, there should be on extended two stage information and consent process. Women should be given information well in advance of being asked for their consent to participate. Informed consent should be sought as close to randomization/ treatment as possible. Consequently, to inform complete and exact information among; exceptional circumstances; informed consent or refusal; written information to keep; contacts, objectives; diagnostic or treatmental procedures; conditions of baby, the right to see the results; ethics,… are the most important elements that should be considered as moral and ethical rights of pregnant research participants.

P-18: Ethical Issues in Embryo Research

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Research on embryo has been surrounded with ethical questions for many years. These questions remain important because of the potential benefits to be gained among; infertility treatment, promotion of contraceptive methods, prevention and treatment of cancers, and prevention and treatment of fetal deficiencies.
In 1974, the U.S Ethics Advisory Board (EAB) was established to review in vitro fertilization (IVF) research protocols and certain protocols for fetal research. In a may 4, 1979, report, the EAB agreed that "the human embryo is entitled to profound respect, but this respect does not necessarily encompass the full legal and moral rights attributed to persons". Consequently in vitro embryo formation for the exclusive research purposes is unauthorized. On the other hand lost IVF obtain embryo tissues could be used in research projects. Also according to EAB recommendation; developing human cells not be sustained longer than 14 days in vitro and safety and efficacy of IVF and embryo transfer from the maternal and fetal views should be considered in fetal deficiency cases by parents satisfaction researches should be limited to treatment protocols and procedures.

P-19: The Relationship between Exercise and Quality of Life in Menopausal Women

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Introduction: The effect of the menopausal transition on quality of life is an important concern. Physical, psychological, and social problems that start with the menopause might influence quality of life in the postmenopausal period. Health promotion for midlife women has gained more prominence, with increasing attention to health-related behaviours, such as

diet, physical activity, and HRT. Regular physical activity might enhance the quality of life in these women.
Materials and Methods: This study was a derived data analysis of the Queensland Midlife Women aged between 45 - 60 years, based postal survey with a questionnaire. Selected women completed a questionnaire, which included measurement of socio demographic factors (age, employment and education attainment) and quality of life measured by SF-36 scale.
Results: ANOVA test determined significant differences in quality of life scores in women who exercise 5-6 times per week as well as participants who exercise 3-4 times per week. (M= 70.354, p=0.000 and M=68.694, p=0.0130) respectively. Women without physical activity had significantly lower quality of life scores than the group who exercised 5-6 times per week.
Conclusion: In conclusion the study showed that exercise was effective in improvement of quality of life in menopausal women. These finding are welcome news for women who their quality of life is affected by menopause.
Keywords: Quality of life, physical activity, exercise, menopause.

P-20: Various Sttitudes about ART Ethics

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The practice of ART has become increasingly complex with advances in methods of ovarian Stimulation, ova and sperm retrieval, fertilization, storage of embryos, and use of donor gametes but from the ethical and religion views ethical issues should be considered as the most essential factor .
Some studies with this approach were done in the world but in our country different aspects of ART and ethical issues must be studied and discussed.
In short, review literature of this matter showed that general population accepted ART ethically more than specialists and academic societies. The reason of these priority in most studied couples shows a meaningful different between two mentioned groups. In other words ethical and moral issues in general population is mostly under influence of confirmed request for child bearing, contrariety educated person with related fields of study are more sensitive and ethics bounded.
Further wide ranging discussion involving the general public and specialist is to be needed.

P-21: The Secret of ART Children

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Introduction: In the early years of gamete donation use, couples often expressed confusion and concern regarding what they might tell their children. In the early 1980 s a number of psychiatrists recommended that no one be told about this truth but some others believed that the couples should be copped with this condition and knowing the truth is children's right.
Materials and Methods: In this study, Review literature was done for clarify this subject.
Results: A study showed that, %74 of parents disinclimated about disclosure to their but in another study %69 of them didn't agree with disclosure.
The parents said that %76 of these children probably will have neutral or positive feeling. Also %90.7 of patient told that their children will be curios and look for finding their real parent. Half of these children will also ask about donor's appearance.
The parents also said that %25 of children will be appreciated about knowing the truth but approximately %30-40 of them will be angry and anxious.
%25 of physicians and reproductive endocrinologist agreed with disclosure to children but %55 didn't agree with this matter.
In summary parents should make decision based on their social, cultural conditions and evaluation advantage and disadvantage of disclosure for them.

Conclusion: Providing a comfortable and good life is necessary for children but is not enough and they have to be satisfied with their life. Culture has a great effect on this copping strategy and special counseling and group therapy can help parents for telling truth to their children.

P-22: Health of Children Born Using Assisted Reproductive Technology– Especially ICSI

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During the past two decades, assisted reproductive technologies (ARTs) have revolutionised the treatment of infertility. ARTs now account for between 1% and 3% of annual births in many western countries and in vitro fertilization (IVF) services are growing worldwide. As ARTs are increasingly used to overcome infertility, there is concern about the health of the children conceived. For many reasons ART might be considered as a risk for problems in the health of children conceived with the help of ART. There is little information about the long-term outcome of children born after ICSI. Evaluation of the health of children born using ART provides important information to clinicians and consumers. In general, the incidence of abnormalities at birth is reassuringly low and children develop normally. Nevertheless, it is important to monitor the safety of ARTs as clinical protocols evolve and new technologies emerge.
The propuses of the present article are assessing the concerns regarding ART (especially intracytoplasmic sperm injection [ICSI]) offspring relate to four general areas of investigation: the obstetrical outcomes of pregnancies resulting from ART, chromosomal abnormalities associated with the offspring of ART pregnancies, congenital malformations of the newborns resulting from the ART procedures, and developmental abnormalities in children born as a result of ART. Therefore, this review article assesses the existing literature to understand better the risks to offspring conceived by this assisted reproductive technique.

Female Infertility

P-23: Repeated Spontaneous Abortion (RSA): A Challenge to Find a Clue within the Cytokine Network

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Background: Recurrent spontaneous abortion (RSA) is defined by "occurrence of three consecutive pregnancy losses before 20 weeks of gestation, with fetus weighing 500 g or less". Approximately one in 300 women worldwide experience RSA. The incidence of RSA among the pregnancies is about 0.5–2%. Although various etiologic factors have been identified, the exact underlying pathophysiologic mechanisms remain elusive in up to 40–50% of cases.
Objective: To undrestand the molecular contribution of Cytokine network genes in the etioly of Recurrent spontaneous Abortion**.**
Materials and Methods: Two key regulatory cytokine genes, the TGF-beta1 and IL-18 were chased and investigated. A case control approach was desgined and various molecular method suchas PCR based screening and PCR direct sequencing were applied.
Results: Whole exon intron screeing of TGF-beta1 gene indicated no significant association between any molecular changes in TGF beta1 gene and in Iranian RSA patients. Role of IL-18 gene promoter variate was also in identical trend to that of TGF-beta1.
Conclusion: The results of the studies on TGF-beta1 together with our recent published information IL-18 did not give any evidence of association of the *TGFB1*-SNPs in the promoter, exons, adjacent intronic regions

and IL-18 promoter regions with recurrent spontaneous abortion in Southern Iranian women.

P-24: Comparison of the Accuracy of Out-Patient Sonohystrography with Transvaginal Sonography for the Screening of Causes Leading to Abnormal Uterine Bleeding

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Objective: To compare of the accuracy of out-patients saline sonohysterography (SIS) with transvaginal sonogrpahy (TVS) for the screening of causes of abnormal uterine bleeding (AUB).
Materials and Methods: 81 patients with AUB were studied. All cases were examined with conventional TVS were further investigated with SIS using saline as contrast medium, finally hysteroscopy was used as the gold standard.
Results: TVS had sensitivity of 72%, specificity of 92%, positive predictive value of 94% and negative predictive value of 65% while SIS had sensitivity of 94.1%, specificity of 95%, positive predictive value of 96% and negative predictive value of 90%. TVS had kappa measure of agreement of 0.60 while 0.86 was reported for SIS.
Conclusion: In our study SIS was more sensitive and specific in diagnosing polyp, myoma and adenomyosis with high positive and negative predictive value. Furthermore results obtained by SIS demonstrate more agreement with that obtained by hysteroscopy rather than TVS.

P-25: Galectin-9: Spatial Expression and Subcellular Localization in the Human Endometrium

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Introduction: Galectins are a family of proteins that bind to galactose-containing ligands. Members of this family have been reported to play a role in a variety of functions that include cell growth, cell adhesion, apoptosis, inflammation, and immunomodulation, all of which are important for endometrial function, as well as implantation. Galectin-9 is one of the very few epithelial markers that are strictly regulated during the menstrual cycle, with a significantly increased expression during the secretory phase. Galectin-9 will be very intriguing to follow up with structural and functional studies in the human endometrium. The objective of this study was to investigate the spatial expression and subcellular localization of galectin-9 in the human endometrium.
Matherials and Methods: We have examined galectin-9 expression in the different area of the surface epithelium in an effort to elucidate the role of galectin-9 in endometrial function. Spatial protein expression and subcellular localization of galectin-9 in uterodumes (pinopods) as well as uterodumes-free area of the surface epithelium during the opening of implantation window were studied by immunogold transmission electron microscopy (TEM).
Results: Our results illustrated that a high level of galectin-9 were localized in uterodumes compare to uterodumes-free area of the surface epithelium during the secretory phase. There was a sharp and significant increase in the galectin-9 expression in uterodumes compare to uterodumes-free area of the surface epithelium.
Conclusion: Based on these findings, we suggest a role for galectin-9 in the human endometrium. The spatial changes in galectin-9 protein in the surface epithelium, combined with the functional aspects of galectin-9 make it an intriguing factor for cell-cell interaction in

the human endometrium, as well as during human implantation.

P-26: Neonatal Birth Weight after Assisted Reproduction

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The reported lower birth weight among infants born from singleton pregnancies induced by assisted reproduction has sparked interest about disturbances of epigenetic reprogramming occurring during these procedures. However, this has relied on the comparison of the birth weights of infants conceived with assisted reproduction to general population data. More appropriate control groups should include naturally conceived pregnancies in infertile women. Analyses of the outcomes of distinctive treatment methods should provide further clues concerning the mechanisms causing the observed differences. The lower birth weight may be caused either by the ovarian stimulation preceding assisted reproduction or by the culture conditions during manipulation of the gametes and embryos.
Between August 1996 and December 2003 the data of all infertile couples were registered prospectively. A formal permission of our ethics committee was obtained. From a total of 930 pregnancies data of 33 deliveries were missing (1.6%) or incomplete (1.9%). In order to examine the effect of the method of conception on the birth weight of singleton infants, the data of 86 twin (9.2%) and 7 triplet pregnancies (0.8%) were excluded together with those of a case with neonatal death and seven cases of infants with major malformations (0.8%). The data of 94 pregnancies (10.1%) were excluded because of premature or delayed delivery.
The data were grouped according to the presence or absence of a previous pregnancy and the infants' birth weight was correlated to one of the following conception modes: in vitro fertilisation (IVF) and/or intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI), transfer of embryos derived from frozen-thawed pronucleate oocytes and natural conceptions.
Children born after assisted reproduction had significantly lower birth weights as compared with those conceived naturally.

P-27: An *In Vitro* Comparative Study of Follicle Stimulating Hormone (FSH) and Activin A Effects on the Maturation of Preantral Follicle-Enclosed Oocytes from Immature Syrian Mice

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Objectives: It was aimed to investigate at what stage; early cultured preantral mouse follicles become dependent on a minimal effective dose of FSH and activin, and analyzed the influence of implementing FSH with different concentrations during in vitro culture.
Materials and Methods: Preantral follicles (90–100 µm in diameter) were harvested from 6-8 week-old Syrian mice and cultured in TCM199 culture medium for 6 days to see the effect of FSH and activin. Activin concentrations in the range of 10-200 ng/ml were used, while 10, 50, 100, 105 and 200 mIU/ml FSH was used in the experiment.
Results: Activin concentration of 100 ng/ml resulted in a significant increase in follicle diameter (170 µm) with the survival rate of 73% as compared to the control (100 µm and 25%, $p<0.0001$). The number of oocytes matured and the percentage of germinal vesicle breakdown (GVBD) was 61 and 70%, respectively as compared to the control (20 and 29%, $p<0.0001$). Follicle diameter (190 µm) and survival rate (85%) increased

significantly in the presence of 100 mIU/ml of FSH as compared to the control (p<0.01). But the administration of activin A+FSH increased the effect of both factors on follicular diameter (205 µm as compared to 100 µm in control, p<0.05). Follicle survival, oocyte maturation and GVBD rates were 91, 81 and 89%, respectively (p<0.05). Withdrawal of activin A and FSH not only decreased the oocyte maturation rate but also resulted in a decreased GVBD.

Conclusion: These results have suggested that exposure to FSH and activin before the formation of antral cavity had positive effect on follicle survival and oocyte robustness.

P-28: Predictive Value of Basal Antaral Follicle on Intra Cytoplasmic Sperm Injection (ICSI) Outcome

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Background: In this era of delayed child bearing, ovarian reserve testing is an increasingly important step in the evaluation of infertile women. Antral follicle counts have become an increasingly used tool in the evaluation of ovarian reserve in women of reproductive age.

Objective: The aim of this study was to evaluate the value of antral follicles for prediction of ovarian response and PR in ICSI cycles.

Materials and Methods: In this prospective cohort analysis, we evaluated the predictive value of the antral follicle on ovarian reserve and pregnancy rate in 200 women undergoing their first ICSI cycle in Mehr infertility institute between Sep.2006 and March.2007. All women underwent a fresh cycle of intracytoplasmic sperm injection with a long protocol with mid-luteal start of the gonadotropin-releasing hormone analog, and antral follicles were counted on cycle day 3 following down-regulation. Inclusion criteria were presence of both ovaries, no history of ovarian surgery, ability to visualize both ovaries on transvaginal ultrasonography, and absence of ovarian abnormalities or ovarian cysts>10mm.Patients were included regardless of their age, concomitant diagnoses or reproductive history. To assess ovarian responsiveness, the pretreatment ovarian ultrasonographic measurements (antral follicle) were compared with respect to patient age, number of mature oocytes, number of embryos, basal serum laboratory values (E2, FSH, and LH), number of ampoules of gonadotropins used, days of stimulation, embryo quality , PR and cancellation rate. Main outcome measure: ovarian response and pregnancy rate (PR). Pregnancy rate was confirmed by measurement of β-hCG in serum after 14 days. After data collection, analysis carried out with T-test, chi squares test and multiple logistic regressions by using statistical software SPSS.10.

Results: During the study period, 200 patients were analyzed. Overall pregnancy rate was in 61/200cycles (30%).The cancellation rate was 14(7%) of all cycles. The mean number of gonadotropin ampula for stimulation (31±10.7 in pregnant vs 36.1±10.5 in non pregnant) influenced on PR (p<0.05). The mean number of embryo transfer related on PR (3.2±0.6 in pregnant vs.2.8±1 in non pregnant) (p<0.05).Other variables weren't statistically significant effect on PR (p>0.05) The mean age of women (30.8±0.4 vs.37.5±1), mean of estradiol levels in 3rd day of period (41.8±45.8 vs.88.9±96.8) and mean of LH level (4.3±4.5 vs.8.1±13.4) influenced on cancellation rate (p<0.05).

Data showed the number of antral follicle had significant effect on ICSI outcome. Pregnancy rate and cancellation rate in patients without any antral follicle were 2(10%) and 8(40%) respectively (p<0.05)

Conclusion: It appears antral follicle counts on 3day of period during ICSI cycle is helpful in predicting ovarian response and pregnancy rate.

P-29: Cesarean Scar Pregnancy Misdiagnosed as Gestation in Bicornate Uterus

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Ectopic pregnancy sited in dehiscent cesarean section scars has a high risk of rupture and bleeding. Attempts at operation therapy frequently end in loss of fertility. We aimed to present a case of CSP that misdiagnosed as a pregnancy in bicornate uterus and leaded to emergency hysterectomy.

Case: A 32- year-old woman, gravid 3, para2 was referred to emergency room with massive vaginal bleeding and preshock state. She had 2 transabdominal sonographic (TAS) examinations. One revealed a viable pregnancy in bicornate uterus in right cornea with diminished amniotic fluid, and the other reported partial mole with viable fetus .The gestational age was 10 weeks and the ß-HCG level was 45000 mIU/ml. She had vaginal bleeding. She had history of two cesarean sections with incision in lower segment of uterus.

She was referred to operation room and suction curettage was performed with the partial mole diagnosis, but fundus was empty. There was a non-molar prominence tissue in the specimen obtained. Hemorrhage continued. Suspicious to perforation or invasive mole, laparotomy was done. Lower segment was bulge with dark blue-red color. An incision in lower segment showed empty fundus and the residue of placenta was present on a very thin isthmus. Bilateral uterine artery ligation and an 8-shaped suture in site of cesarean scar and Macdonald cercelage in cervix were done. The folly catheter was inserted in cervix. Bleeding was still severe.

Ultimately hysterectomy was done. There was a heamatoma on the anterior wall of cervix. Pathologic exam showed a villi band implanted in myometrium of isthmus surrounded by fibroid tissue. Packed-cell & whole blood were infused. Six hours after surgery, respiratory distress was present. She was referred to ICU. After 2 days of ICU and 8 days of ward admission, she was discharged with a good general condition.

P-30: The Effect of Prednisolone Therapy on Delivery Outcome in Women with the Past History of Idiopathic Spontaneous Abortion

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Introduction: About 0.3% of all women suffer from Idiopathic Spontaneous Abortion (ISA) which is defined as 3 continuous miscarriages without any pharmacoceutical intervention or instruments. Immunologic factors are responsible for more than 50% of ISAs. The aim of this study was to assess the affect of prednisolone on delivery outcome in women with ISA.

Materials and Methods: Of all patients with the history of ISA who were followed up in our clinic, 50 were included in the study and filled up the questionnaire and satisfaction form. Women with determined causes of ISA were excluded form the study. After the first βHCG positive result, women were divided into two groups: 25 for placebo and 25 for prednisolone therapy who took 10 mg prednisolone per day. Uterus sonography and glucose tolerance test were performed during pregnancy.

Results: The mean age of patients was 27 years old and 20% had positive family history of ISA. In prednisolone group the abortion, preterm and term labor rates were 12%, 22% and 56%, compared to 20%, 32% and 48% for placebo group respectively. There was no statistically significant difference in delivery outcome between 2 groups.

Conclusion: Although 56% of patients of prednisolone group experienced normal term delivery compared to 48% for placebo group, prescription of low dose prednisolone did not improve delivery outcome. Also prednisolone didn't increase the risk of pregnancy induced diabetes mellitus, hypertension and intrauterous growth retardation. The effect of prednisolone on delivery outcome needs more researches.

P-31: Comparison of Twin Pregnancy Outcome after Assisted Reproductive Technology with and without Embryo Reduction

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Background: Spontaneous occurrences of multifetal pregnancies always have been a medical problem.
The risks of perinatal morbidity and mortality and maternal morbidity increase with enhancement of the number of fetuses. In our prospective experimental study, the outcome of twin pregnancy after Assisted Reproductive Technologies (ART) with and without Multifetal Pregnancy Reduction (MFPR) is compared relating to perinatal and maternal complications.
Objective: The aim of this study was to compare the gestational age at delivery, birth weight, and other complications of surviving twins following MFPR to those in a control group of non-reduced twins.
Materials and Methods: In this prospective experimental study, from infertile couples who were referred to Isfahan Fertility- Infertility Center (IFIC) and were candidate for ART (Invitro Fertilization or Intra Cytoplasmic Sperm Injection), 30 couples who have had twin (control group) and 35 couples with quadruplet or higher order pregnancies (experimental group) were selected. In cases with experimental group MFPR was done, and pregnancy outcome-miscarriage, premature labor, Premature Preterm Rupture of Membranes (PPROM) and Pregnancy Induced Hypertension (PIH)-were compared between two groups.
Results: Distribution of complications in experimental vs. control groups was as follows: miscarriage: 23.3 vs. 16 .7%, premature labor: 15.7% vs. 13.3 %, pregnancy induced hypertension: 13.3% vs. 16.7%, abruption: 6.7% vs. 6.7%, and premature preterm rupture of membranes: 23.3% vs. 26.7 %. Mean neonatal weight at birth (2239 vs. 2240 gr) and mean gestational age at delivery (33.5 vs. 34.1 w) were similar. The differences between two groups were not statistically significant (p>0.05).
Conclusion: MFPR during early pregnancy is a safe, effective and simple operative for the purpose of reducing perinatal and maternal complications.

P-32: The Effect of Temperature on the Outcome of Intrauterine Insemination

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Objective: The aim of the study is to determine the effect of temperature on intrauterine insemination (IUI) outcome.
Design: Prospective study Subjects and methods: In the study comprised 371 couples who underwent 568 IUI treatment cycles. The IUI cycles were done in two different time of Novamber2005 to September 2006 , 274 IUI cycles were performed in the cold weather, and 294 IUI cycles were performed in the hot weather. In the cold weather, the minimum of temperature was 3°C to 17°C and the maximum was 13°C to 27°C, and the minimum of temperature in the hot weather was 25°C to 32°C and the maximum was 43°C to 50°C. The selected infertile couples were male factor infertility with abnormal parameters of semen or sexual dysfunction, unexplained infertility with history of 3 to 6 times induction ovulation or poor post coital test, and ovulatory factor infertility with history 3 to 6times induction ovulation. Patients characteristics (age of women and ovulatory cycles), and the parameters of semen (Total motile sperm count and percent of normal morphology) in two part of time were matched. All of women were stimulated with clomiphen citrate and at least 75IU HMG irrespective of whether they were ovulatory or anovulatory and HCG(5000IU,IM) was administrated when at least a follicle reached a mean diameter of 18mm. Raw semen

processed for IUI using swim up. A single IUI was performed 36 hours later. The positive result was based on serum B-HCG. The result of IUI was compared by chi-squre test. Statistical significance was set at P<.05.

Results: Among 274 IUI cycles in the cold weather, 24 patients were pregnant, the overall pregnancy rate (PR) was 8.75% per cycle, and among 294 IUI cycles in the hot weather 18 patients were pregnant, PR was 6.12%per cycle. (P=0.23) Therefore, there was not significant correlation between temperature and the outcome of IUI.

Conclusion: The finding of the study shows IUI assisted conception rate is not influenced by temperature. Therefore, the rate of temperature of weather is not any restriction for performing IUI; on the other hand, it appears the spontaneous conception is an independent factor from the temperature.

P-33: Risk Factors for IUD Failure: Results of a Large Multicentre Case-Control Study

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Objective: This study was conducted to identify the risk factors for intrauterine device failure.

Materials and Methods: A retrospective case-control study was carried out between 1999 and 2002. Cases (women with an IUD and a confirmed pregnancy) and controls (women with an IUD who were not pregnant) were recruited by gynaecologists. An anonymous questionnaire was filled in during the consultation, with specific items regarding any type of drugs used before the predicted fertile period for cases and within the cycle which ended in menses for controls.

Results: Two hundred and sixteen cases were compared with 657 controls. Age was associated with intrauterine device failure, with a significantly lower failure risk in women older than 35 years. A significant relationship was observed between a history of IUD expulsion and IUD failure risk (age-adjusted odds ratio 3.31, 95% CI: 1.40-7.81). No relationship was observed between risk of IUD failure and gynaecological background (fibroma, polyps, miscarriage), nor with any type of medicine taken by the woman.

Conclusion: This study is clearly reassuring as we found that anti-inflammatory drugs and any other medicines taken by the woman were not implicated in IUD failure. Only a history of previous IUD expulsion was found to be a risk factor for failure, indicating that these women should have regular medical and echographical follow-up. Comparing the efficacy rate of various types of IUD, we found a clear advantage for levonorgestrel-releasing devices.

P-34: Ultrastractural Effects of Superovulatory Drugs on Human Glandular Endometrial Epithelium at LH + 6

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Background and objective: Using stimulatory drugs such as; GnRH, HMG and HCG for induction of superovulation is a common procedure in ART. Regarding the principal role of endometrial glandular secretions on implantation, the aim of the present study is to investigate the ultrastractual effect of stimulatory drugs on glandular epithelium at LH+6.

Materials and Methods: For this purpose, biopsies were obtained at LH+6 from infertile women who were underwent ART protocol and fertile women as control, using pipelle suction (Rochet International). The specimens were processed for electron microscopy and morphometric studies. The data obtained from

morphometric studies were analyzed using student t-Test.

Results: Electron microscopy revealed that glandular epithelial cells in case group, in comparison to control group, had large vesicles containing mucoid material. The vesicles were protruded into the lumen of the glands as secretory substance. The nuclei of the cells were large and euchromatic, the cytoplasm contained giant mitochondriae and well developed rER. Morphometric studies showed that Vv of nucleous to cytoplasm, rER to cell and mitochondriae to cell were significantly higher ($p<0.05$) in case group than the controls.

Conclusion: It is concluded that superovulatory drugs both stimulates folliculogenesis and glandular epithelial maturation in ART cycles.

Genetics

P-35: Meiotic Competence and DNA Damage of Porcine Oocytes within Follicles after Exposure to Elevated Temperature

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Introduction: Reproductive performance of cattle reduced during hot seasons. Adverse effects of heat stress on follicular growth, corpus Luteum function, expression of estrous behavior, Superovulatory response, quality of embryos and fertility are documented in cattle. Direct exposure of bovine oocytes at the GV stage to 41oC for 12 hours reduced their ability to complete nuclear maturation and development after maturation. Moreover, some studies have shown DNA fragmentation and cytoskeleton disruption of oocyte after exposure to heat stress during maturation. The objective of this study was to investigate the effects of exposure time of porcine oocytes within follicles to an elevated temperature (41°C) on their meiotic competence and DNA damage.

Materials and Methods: A total of 80 porcine ovaries, collected from slaughterhouse and transported in 35oC physiological saline to laboratory within 3 h after sloughing, were stored in physiological saline at 41°C for 0 h (control; n=20), 0.5 h (n=20), 1.0 h (n=20) and 1.5 h (n=20). After exposure of ovaries to the elevated temperature, COC's were collected by slicing of the antral follicles (2-8mm diameter) and cultured for 44 h in a modified North Carolina State University-37 (NCSU-37) solution under humified environment with 5% CO2 and 95% Oxygen. Meiotic stage and DNA damage of oocytes were analyzed using combined technique for simultaneous nuclear staining and and terminal deoxy nucleotidyl transferase (TdT) nick-end labeling (TUNEL) by a modified procedures previously described by Otoi et al. To assess DNA damage of oocytes before and after maturation culture, the numbers of nuclei labeled by TUNEL were counted. Data were analyzed by ANOVA after arc sin transformation using statview software.

Results: The proportions of oocytes reaching metaphase II (MII) significantly decreased (59.1±5.0, 38.4±9.1, 24.6±3.9 and 15.8±3.5) with increased exposure time (0 h, 0.5 h, 1.0 h and 1.5 h), respectively ($p<0.05$). When the ovaries were exposed for 1.5 h (46.6±16.2), there were significantly more ($p<0.05$) MII-stage oocytes with fragmentation of nuclear DNA- compared with control oocytes (10.5±2.8). The exposure time of ovaries to 41°C had no effect on the proportions of oocytes with DNA fragmented nuclei before maturation culture ($p>0.05$), but influenced the proportions at the end of maturation culture ($p<0.05$).

Conclusion: These results indicate that the meiotic competence and DNA damage of porcine oocytes are dependent on the duration of ovaries exposure to the elevated temperature. Moreover, the occurrence of DNA damage of oocytes becomes more apparent after maturation culture than before the culture.

P-36: Developmental Congenital Ocular Tumors: A Rare Clinicopathologic Report of Globe Xantoma in 8 Month Child

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Objective: The authors report the case of an 8-month-old female who presented with an expansive retinal detachment in the right eye, mimicking retinoblastoma.
Materials and Methods: Eight Month old girl presented to the Eye Center of Nikookari Hospital , Tabriz , Iran , for unilateral blind eye leukocoria with positive marcus gun ,exophitic tumors , peripheral vasculitis, total.
Results: Enucleation were performed on right eye and macroscopic finding consist of globe 22 millimeter diameter ,classic cut section show retinal detachment with fusiform gelatinous hard clear yellowish trancelucent vitreous material subretinal yellow masses gross appearance of lesion mostly show chemical nature as xantocell and multifocal small distribution microscopy reveals macrophages reaction granoloma formation pathogenesis seems release of granules of pigmented layers phagoctosis became this pattern, no retinoblastoma seen.
Conclusion: Diagnostic Pittfal in clinical finding Are: Leukokoria, Early age Congenital or from birthday, Attention to Pathogenesis.

Stem Cells

P-37: Stem Cells in Ophthalmology

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Objective: To report the Stem cells offer new potential for tissue replacement therapy and delivery of therapeutic agents, and their use is rapidly being applied in a variety of Ophthalmology fields and severe limbus stem cell insufficiency after trabeculectomy with subconjunctival injection of mitomycin C.
Materials and Methods: Nine consecutive glaucoma patients (12 eyes) underwent penetrating trabeculectomy that included subconjunctival injection of 0.1 to 0.2 ml of mitomycin C (0.2 mg/ml) at the 12 o'clock position.
Results: All patients with a follow-up time of >15 months (n=6 eyes; 48%) experienced marked ocular surface problems that included corneal thinning (n=2) and scleral melting (n=1). Five patients (42%) with a follow-up time of <14 months did not show complications that were attributable to the subconjunctival application of mitomycin.
Conclusion: Because limbal stem cell deficiency may be a late complication of subconjunctival mitomycin C injection, subconjunctival injection of mitomycin C may be avoided in routine antiglaucomatous filtering surgery.

P-38: The Effect of 5-Azacytidine on Bone Marrow Stromal Cells Differentiation into the Cardiomyocyte-Like Cells

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P-39: Attachment and Spreading Kinetics of Stem Cells Using Optical Waveguide Lightmode Spectroscopy

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Cell-adhesion is an active process, carried out via receptor-ligand like interactions between cell surface adhesion molecules and the extracellular matrix. Initial cell surface

reactions may trigger multiple responses, which in turn result in either spreading or detachment of the cell. The set of adhesion and attachment molecules mediating the adhesive behaviour of stem cells and the kinetics of their interactions are largely unknown.
Traditionally optical waveguide light mode spectroscopy (OWLS) has been used to quantify thin film material deposition at the solid sensor surface (within the sensing depth ~150 nm) and has been used to measure the kinetics of cell adhesion and spreading on predefined surfaces. Due to the relevant shallow penetration depth cell focal adhesion events are mainly detected using OWLS whilst the majority of the cellular mass remains beyond the detection field. Using a novel waveguide design we are able to tune and predefine the probing depth and obtain more detailed information on the morphology of the cells and their behaviour at the surface.
Substrate-stem cell investigations are important for therapeutic applications (such as 3D tissue growth) and non-therapeutic, e.g. cell development for therapy, disease modelling and protein production. We have therefore investigated the attachment and spreading of precursor cells on various surfaces ranging form glycoprotein to nanotextured metal oxides using OWLS. We suggest possible future non-therapeutic applications for these substrates.

P-40: OCT-4, an Embryonic Stem Cell Marker, is Gighly Expressed in Bladder Cancer

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Introduction: OCT-4 (also known as POU5F1) is a key regulator of self-renewal in embryonic stem cells. Regarding the new cancer stem cell concept, the expression of such genes is potentially correlated with tumorigenesis and can affect some aspects of tumor behavior, such as tumor recurrence or resistance to therapies. Although OCT-4 has been introduced as a molecular marker for germ cell tumors, little is known about its expression in somatic cancers.
Materials and Methods: Here, we have investigated the potential expression of OCT-4 in bladder cancer. We used semiquantitative RT-PCR to examine the expression of OCT-4 in 32 tumors, 13 apparently nontumor tissues taken from the margin of tumors and 9 normal urothelial tissues. The expression of OCT-4 at protein level was further determined by Western blotting and immunohistochemical (IHC) analysis.
Results: OCT-4 expression was detected in almost all examined tumors (31/32), but at much lower level ($p < 0.001$) in some nonneoplastic samples (6/22). A significantly strong correlation of 0.6 has been observed between OCT-4 expression and the presence of tumors ($p < 0.001$). Western blot analysis further confirmed the expression of OCT-4 in tumor biopsies. According to IHC results, OCT-4 is primarily localized in the nuclei of tumor cells, with no or low immunoreactivity in nontumor cells.
Conclusion: Our study demonstrated, for the first time, the expression of OCT-4 in bladder cancer and a further clue to the involvement of embryonic genes in carcinogenesis.

P-41: Nuclear and Total Proteome Analyses of Monkey Embryonic Stem Cell Differentiation

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P-42: Application of Proteomics to Identify Human Embryonic Stem Cell-Associated Proteins

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P-43: Differentiation of Murine Embryonic Stem Cells into Endothelial Cells

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Objective: In this investigation murine embryonic stem (ES) cells were used to study endothelial cell development.
Materials and Methods: Murine ES cells (CCE cell line) exposed to Alpha-MEM medium containing 10% FBS for 4 days and then cultuted in endothelial basal-2 medium containing vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF) and 5% FBS.
Results: The cells were assumed a relatively uniform endothelial cell morphology and could be propagated and expanded. When placed in Matrigel, these murine ES cell–derived endothelial cells (MESDECs) formed capillary-like structures characteristic of endothelial cells. Immunohistochemical and RT-PCR analysis of differentiated cells showed that they take up acetylated low-density lipoprotein (LDL), express flk-1, CD31, CD34, Ac133, tie1 and tie2genes, and bind the BS-lectin.
Conclusion: MESDECs provide a novel means to examine the mechanisms of endothelial cell development, and may open up new therapeutic strategies.

P-44: Human Umblical Cord Blood-Derived Unrestricted Somatic Stem Cell, A New Human Feeder Layer for Expand Embryonic Stem Cell *In Vitro*

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Mouse embryonic fibroblast (MEFS) have been use support the growth of mouse embryonic stem cells (mESc) and human embryonic stem cells (hESCs). Prolonged propagation of mESCs is currently achived by coculture with MEFs servis as feeder cells. The presence of uncharacterized rodent cells or crude extracts imposes a risk to the clinical applications of hESCs or mESCs, Culture-expanded.
Human cord blood derived unrestricted Somatic stem cells (USSC) from multiple donors were Used as feeder Cells to support growth of he C4 mES cell under a Embryonic stem cell culture. Mouse Es cell colonies cultured on inactived hUSSCs am plified>600-fold during 30-day continuous culture (in them passage). The expanded mES cells displayed the unique morphology and molecular markers characteristic of undifferentiated mEs cell as observed when thay were cultured of MEFs.
Thay expressed the transcription factor oct-4, a membrane alkalin phosphatase, and the stage–specific embryonic antigen (SSEA)- 1 but not the SSEA4 marker. Expanded, mEs cells on hUSSCs retained unique differentiation potential in culture and a normal diploid karyotype. Well–studied hUSSCs may provide a clinically and ethically feasible method to expand hEs cells for novel cell therapies.

P-45: A New Human Feeder Layer for Expand Embryonic Stem Cell *In Vitro*

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Introduction: Mouse embryonic fibrblast (MEFS) have been use support the growth of mouse embryonic stem cells (mESc) and human embryonic stem cells (hESCs).
Prolonged propagation of mESCs is currently achived by coculture with
MEFs servis as feeder cells. The presence of uncharacterized rodent cells or crude extracts imposes a risk to the clinical applications of hESCs or mESCs.
Materials and Methods: Embryonic stem cell wear expanded using human USSC, and then expression of CD146, CD29, CD49, VEGFR2, FLK1 were evaluated by flowcytometry and expression of Stat3, BMP4, REX1, Oct4, SOX2, Nanong, Brachyury, Tert, LIF, LIFR Fgf4, were evaluated by RT-PCR and protein expression of Oct4 were evaluated by Immunohistochemestry.
Results: Mouse Es cell colonies cultured on inactived hUSSCs am plified>600 – fold during 80-day continuous culture (in 30 passage). The expanded mES cells displayed the unique morphology and molecular markers characteristic of undifferentiated mEs cell as observed when thay were cultured of MEFs.
Thay expressed oct-4, BMP4, REX1, Nanong, Brachyury, Tert, LIF, LIFR, and but not SOX2, Stat3, Fgf4.
Expanded, mES cells on hUSSCs retained unique differentiation potential in culture and a normal diploid karyotype.
Conclusion: Our resolt indicated that co-culture of ESC on cord blood stem cell(USSC) significantly maintance ESCs in the undifferention stat.
Well–studied hUSSCs may provide a clinically and ethically feasible method to expand hEs cells for novel cell therapies.

P-46: Comprehensive Effect of Different Growth Factors for Differentiation of Rat Bone Marrow Mesenchymal Stem Cells into Hepatocyte-Like Cells

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Objective: To understand which growth factors/cytokines can affect in differentiation of bone marrow mesenchymal stem cells (BMSCs) into hepatocyte –like cells.
Materials and Methods: Bone marrow mesenchymal stem cell (BMSCs) isolated from female rat (3-4 weeks) and was used after (3rd-4th) passage in all the experiment. BMSCs were induced to differentiation into hepatocyte –like cells by (HGF,OSM, EGF, Dexamethasone) the experiment comprise 5 **Group:** Group A(HGF,EGF, Dexa) , group B(HGF,EGF), group C(HGF,EGF,Dexa ,and OSM) , group D(HGF,OSM,EGF) ,group E (OSM,Dexa) and undifferentiated MSCs were used as the negative group. the expression of hepatic markers (AFP, Alb ,Anti hepatocyte) were detected by immunofloresence assay, and RT-PCR after induction in each group at different times after induction. The expression of Albumin and SGOT, SGPT were analyzed by Eliza .
Results: Immunocytochemically staining revealed positive AFP and Alb in all groups except control group, similar the finding of expression of Alb, CK19 AFP, mRNA by RT-PCR. Expression of Alb, SGOT, SGPT in group C after added OSM (7-14days) were higher than other group and expression of SGOT, SGPT after time induction respectively increased in 28 days.
Conclusion: Adult rat bone marrow in the presence of HGF, EGF, OSM and dexamethasone significantly induced Albumin production and SGOT, SGPT and induced into hepatocyte differentiated invitro and may be useful for cell transplantation teraphy.

P-47: Induction of Arterial Calcification in the Vascular Smooth Muscle Cell Cultures by a Potent Calcium-Channel Blocker Drug

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Introduction: Arterial calcification, a regulated process similar to ossification in bone, is common in atherosclerosis. A subpopulation of bovine aortic media cells, have osteoblastic characteristics and form spontaneously mineralized nodules in vitro. To assess whether Benidipine Hydrochloride as a potent calcium-channel blocker modulates arterial calcification, the effect of this drug on bovine vascular aortic smooth muscle cells for formation of calcified nodules and alkaline phosphatase activity in the culture medium was determined.
Materials and Methods: Bovine thoracic aortic media cells were cultured from explants sectioned from luminal face of aortic media. Cell clones were trypsinized before formation of nodules, plated in 12-well tissue culture dishes at a density of 16000 cells/cm_2, and were grown for 21 days. Twenty-four hours after seeding, different concentrations of BD (0, 0.01, 0.1, 1, 10 nmol/L) were added to the cultures. The specimens after 21 days of growth were stained for mineral deposition by Von Kossa method. Also the cultures were test for alkaline phosphatase activity after 14 days of growth up. Data are expressed as mean ± Standard Deviation (SD). Significance of treatment- mediated differences was determined by Student's t-test and P value of <0.05 was considered significant.
Results: Twenty-one days of treatment in comparison with control cultures, resulted in a significant increase in number of calcified nodules visualized by von Kossa staining, as well as by increase in alkaline phosphatase activity, a marker for osteoblastic differentiation and decrease in cell number in a dose dependent manner.
Conclusion: These results indicate that treatment with Benidipine Hydrochloride treatment in long term may contributes to vascular calcification.

P-48: Effect of Silk Proteins on Osteoblast Differentiation of Rat Bone Marrow Stromal Cell Cultures

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Introduction: Silk fibroin is a suitable protein for osteogenesis by inducing markers of bone formation in the cultures of osteoblasts, so we examined the ability of this protein to induce mineralized nodules in the rat bonemarrow stromal cell cultures.
Materials and Methods: Bone marrow stromal cells obtained from 4 to 6 weeks old Spruge-Dawely male rats were grown in primary culture for seven days and then subcultured for 21 days. The secondary cultures were done on either silk fibroin-coated polystyrene plates or free-silk fibroin ones. After 21days of grow up, the cultures were examined for nodule formation by scanning electron microscopy, for mineralization by alizarin red S staining and for expression of gene markers of osteoblast maturation byreverse transcription PCR (RT-PCR).
Results: The stromal cells were observed to form three-dimensional nodules when cultured on the silk fibroin and compared to the stromal cells cultured on the free-silk fibroin polystyrene plates, where no nodules were observed in the time-frame studied. These nodules were also found to be mineralized and expressed the gene markers of osteoblast.
Conclusion: Silk fibroin could serve as suitable inducing factor by stimulating stromal cell differentiation to form mineralized nodules.

P-49: Differentiation Unrestricted Somatic Stem Cells (USSCs) into Cartilage

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Introduction: Stem cells research is an important part of biotechnology that could lead to the development of new therapeutic strategies. Here a new , intrinsincly pluripotent population stem cells from human cord blood stem cells (USSCs) is described .this rare

population grows adherently and differentiated into chondrocyte.
Materials and Methods: For chondrogenic differentiation, a micromass culture system with poly-L-lysin involves the clustering of cells via ionic cross- linking was used. USSCs were cultured in DMEM low glocuse supplemented with antibiotic, dexamethasone, ascorbic acic-2- phosphate, sodium pyrovate , ITS+ premix and TGF-β. The result were analysed by flowcytometry, the mRNA expression of various chondrocyte specific gene analysed using RT-PCR), in days of (21), and expression of collagen II was determined by Alcian blue staining and immunohistochemistry.
Results: Taken together, these results suggest that the combination of differention factor and poly-L-lysin induced USSCs into chondrocyte.
Conclusion: Cultured USSCs differentiated in to a chondroblast cell lineage are potential sources for cell transplantation for Rumatoid Arthrities.

P-50: Selective Growth of Epithelial Basal Cells from Human Prostate in a Three-Dimensional Organ Culture

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A three-dimensional organotypic culture method has been developed for selectively growing epithelial basal cells from human benign prostate.
Tissue fragments were cultured on sponges for several weeks and the viability of luminal and basal epithelium and cellular responses to 4,5a-dihydrotesterone (DHT) stimulation were studied.
The gland tissue could be successfully maintained showing preservation of ducts and lobules as in vivo. Without DHT, a progressive hyperplasia of basal cells was observed: these cells proliferated with retention of the lumen or forming nests with squamous differentiation, lining the surface of the fragment and migrating to the underlying sponge.
In contrast, secretory cells disappeared. Epithelial cells isolated from long-term cultures showed a typical basal cell-mmunophenotype. DHT-treated tissues maintained a much higher percentage of luminal cells than untreated tissues.These systems allow the study of proliferation and differentiation of basal cells within their natural microenvironment as well as prostate pathobiology.

P-51: Ultrastructural Features of Apoptosis in Gametogenic Cells Exposed to Environmental and Chemical Factors

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Background: Apoptosis plays a principle role in controlling germ cell populations. Several environmental and chemical factors could affect apoptosis. One of the critical methods for detection of apoptosis is studying their morphological features.
Objective: The aim of the present study is to investigate ultrastructural features of apoptotic cells produced by electromagnetic field, cyclophosphamide and busulfan in gonads.
Materials and Methods: For this purpose adult male mice received a single dose of Cyclophosphamide, busulfan and adult female mice were exposed to 3mT electromagnetic field for 4 months. The mice in all groups were sacrificed, testicular and ovarian specimens were fixed in glutaraldehyde or formaldehyde and proceed for electron microscopy and TUNEL staining.
Results: TUNEL positive cells were found among spermatogenic and granulose cells but oocytes were not positive. Electron

microscopic study revealed that apoptotic spermatogenic cells mostly had a dilated nuclear membrane; highly condensed nuclei and or nuclear chromatin were disintegrated. Oocytes in atretic follicles which undergo apoptosis had an irregular\ and shrunken nuclei with dilated nuclear membrane.
Conclusion: It is concluded that unlike the apoptotic cells in other tissues, chromatin margination and formation of crescent shape chromatin is not common in gametogenic cells, and morphological features of apoptosis, in these cells are not typical.

P-52: Autologuse Serum Could Significantly Improve the Viability and Proliferation Index of Rat Mesenchymal Stem *In Vitro*

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Introduction: 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 diseases. 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.
Materials & Methods: 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 fetal bovine serum for three successive subcultures during which the morphology, viability and growth kinetic of the cells were closely examined. Passaged-3 cells from either group were easily differentiated into osteoblastic, chondrocytic and adipocytic cell lineages. 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.
Results: passaged-3 MSCs from FCS group seemed to be somewhat shorter and broader 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 significantly a higher growth than cells cultivated in FBS medium. 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 ($p<0.004$).
Conclusion: Taken together, 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.

P-53: Alpha-Fetoprotein Promotes Growth Activity of Three Bone Marrow Hematopoietic Stem Cell Subpopulations *Ex Vivo*

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Introduction: Recently it was shown dependence of fetus hematopoiesis activity from alpha-fetoprotein (AFP) level production

when its higher support correlated with hematopoietic hearths presence and their activities in fetal liver. However, the regulatory-modification role of AFP in growth function of hematopoietic stem cells (HSCs) is still unknown. Main direction of these investigations was study of AFP induction on three mice bone marrow HSC subpopulations growth activity ex vivo.
Materials and Methods: Proliferation and cell cycle were analyzed by flow cytometry using BrdU Flow Kit, propidium iodide and anti-Histone H3(pSer28)-APC antibody. Total protein synthesis level was studed due to 3H-Leu incorporation. Metabolic activity analyzed by piruvate level, citrate synthase activity and ATP level in enzymatic method using Pyruvate Assay Kit, Citrate Synthase Assay Kit and ATP Bioluminescence Assay HS Kit II.
Results: It was evidence that really AFP displayed properties of growth factor for three HSC subpopulations. AFP specifically stimulated three major cell processes involved in maintenance of cell growth functions: proliferation, protein synthesis and metabolism. Besides, AFP modulates HSCs cell cycle progression into synthetic phase domination. Theses AFP growth-regulatory effects on HSCs were strictly AFP-depended, direct and had a strong generation kinetic. More than that none of albuminoid gene family members even serum albumin which is the protein more closely stands to AFP by the structure and nonspecifically interacts with HSCs surface reliable effected on growth potential of HSCs by all investigated parameters. On the other hand, polyclonal antibody against AFP that not interacted with serum albumin fully blocked all AFP growth-regulatory effects.
Conclusion: AFP directly takes part in mechanisms of growth regulation and cell cycle progression of three phenotypically different of mice bone marrow HSC subpopulations, and most likely that AFP play a role of HSCs specific polyvalent growth factor ex vivo and possible in vivo.

P-54: Comparison of Potential Proliferation and Growth of Mouse Urothelial Cells Cultivated on three Individual Matrixes Include Human Amniotic, Peritoneal and Omentum Membranes

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Introduction: Tissue engineering methods can be applied to regenerate diseased, or congenitally missing, urinary tract tissues. Urinary system cell cultures must be established in vitro and adequate matrixes, acting as cell carriers, must be developed. The aim of the present study was to compare the proliferation quality of mouse urothelial cells on three matrixes human amniotic, peritoneal and omentum membranes we compared the morphology, colonization and cell layer in our investigation.
Materials and Methods: Mouse urothelial cells were isolated from normal mouse bladder by treatment with collagenas IV. Then, single urothelial cells were cultured (105 cells/ml) on three matrixes include human amniotic, peritoneal, omentum membranes and collagen matrix as a control group. The pattern of growth and asymmetric unit membrane (AUM) formation were analyzed by Histological examination and immunocytochemistry on the detached urothelial with pan- cyto keratin and uroplakin III respectively.
Results: The immunocytochemistry of cultivated urothelial cells showed the expression uroplakin III of the asymmetric unit membrane (AUM) (indicating cell differentiation) and pan-cytokeratin (an urothelial cell marker) antibodies were used to confirm urothelial cells phenotype. Morphological analysis showed that ammiotic membrane is the best matrix for proliferation, growth and attachment of urothelial cells and surprisingly we obtained up to 4 cells layer in Am and 1-2 layers on peritunm .On the other hand, omentum was not a good material for culture, growth and distribution of the urethelial cells (due to its big pores). Meanwhile the distribution of the urothelial cells didn't lead to a flat layer.

Conclusion: These results showed that amniotic membrane can be used to act as a cell carrier for cultured urothelial cells its wonderful properties such as having various growth factors, availability and cost effectiveness make it's a unique biological matrix for urothelial culture . Also, such a cell-matrix construct could be applied in reparative surgery of urinary system.

P-55: Mature Chondrocyte Promotes Cartilage Differentiation of mMSCs in Co-Culture Systems

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Introduction: Co-culture systems would be considered as an alternative way for cartilage differentiation of MSCs. It has previously been shown that chondrogenesis of MSCs can be improved in co-culture systems with other cells. One important limitation of such systems is that differentiated mesenchymal cells could not be applicable for transplantation. In this context, the use of mature chondrocytes as a co-culture cells could theoretically solve this problem since these cells as a native cartilage cells can without of any concern be transplanted along with those differentiated stem cells. Moreover, cartilage cells may provide stronger chondro-inductive effects than the other cells. Despite of such putative advantages, there is no report regarding chondrogenic potential of this co-culture system which is the subject of the present investigation.
Materials and Methods: The MSCs were isolated from the bone marrow of 4-6-weeks old NMRI mice by low-density primary culture system. Chondrocytes were obtained from rat costal cartilage. To prepare co-culture systems, passaged-2 MSCs and primary-cultured chondrocytes were mixed in various ratio including 1/1, 1/2 and 2/1 (chondrocytes/ MSCs) and cultivated in a micro mass culture system for three weeks, in the end of which the different groups were examined by toluidine blue staining for metachromatic cartilage and RT-PCR analysis for some murine cartilage markers.
Results: Co-culture results indicated that in the group of 1/2 ratio, the abundant of purple metachromatic matrix containing murine collagen II, X and aggreacan were produced whereas in other two groups (1/1 and 2/1 ratio) the matrix were rarely observed. According to our results, cartilage differentiation in the group 1/2 ratio was even intensive than the routine micro mass culture system for cartilage differentiation.
Conclusion: taken together, considering strong chondrogenic effects of mature chondrocyte on MSCs it seems that this strategy could be regarded as a MSCs cartilage differentiation.

P-56: The Structure of Cartilage Generated by mMSCs *In Vitro* Somewhat Differs from that of *In Vivo* Cartilage

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Introduction: MSCs could be considered as an appropriate substitute for mature chondrocyte in cell therapy for cartilage defects. Therefore, one important step is providing the condition in which the cells can differentiate into mature cartilage. Using micro mass culture system, MSCs could be differentiated into cartilage, but the exact final structural differentiation of MSCs into cartilage tissue, being subject of the present study, has not yet been reported. In this study mMSCs were differentiated into cartilage, and the structure of differentiated cartilage compared to the cartilage of the costal rib.
Materials and Methods: 6-8-weeks old NMRI mice were sacrificed and MSCs resident in their bone marrow were isolated by low density primary culture system. For chonderogenic differentiation, 200000 passaged-2 spindly-shaped cells were pletted and cultured in chondrogenic medium for 3 weeks, in the end of which the differentiation examined by RT-PCR analysis. To compare

the structure of differentiated tissue with that of natural cartilage, the cartilage differentiated from MSCs and the cartilage obtained from the same murine rib were prepared for transmission electron microscopy (TEM) study.

Results: RT-PCR analysis showed that a considerable amount of cartilage specific genes including collagen II, X and agreacan are produced in differentiated cells, indicating that the cells differentiated into chndrocytic cell lineage, but when the TEM images were observed it seemed that the in vitro cartilage are structurally somewhat different from in vivo cartilage. In contrast to costal cartilage cells, differentiated cells possessed a euchromatic nucleus and a rich-organelle cytoplasm, a typical feature of the cells in active state. Furthermore the matrix in differentiated cartilage seemed to be more fibrillar than of that in costal cartilage tissue.

Conclusion: Taken together, our results indicated that cartilage tissue differentiated in micromass culture system is not exactly similar to in vivo cartilage in term of some ultra structural features.

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