Abstracts of

Royan International Twin Congress

9th Congress on Reproductive Biomedicine
4th Congress on Stem Cell Biology & Technology

27-29 August 2008

ROYAN INSTITUTE

Tehran, Islamic Republic of Iran

Yakhteh Medical Journal (The Cell)

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.

1. Types of articles

The articles published in *Yakhteh Medical Journal (The Cell)* fall into the following columns:

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.

B. Review articles are the articles by well experienced authors and those who have Excellencies in the related fields. These are usually solicited by the editors, but we will consider unsolicited materials too. They will undergo editorial process except solicited articles. The corresponding author of the review article must be one of the authors of at least three articles appearing in the references.

C. Case reports are published only if the report is of exceptional interest.

D. Short Communications are the articles containing new findings. Submissions should be brief reports of ongoing research.

E. Letters to Editor are comments from our readers on recently published articles.

F. Images in Biology should focus around a single case with an interesting illustration such as a photograph, histological specimen or investigation. Color images are welcomed. The text should be brief, and have a teaching point.

2. Submission Process

A. Cover letter: Each article should be accompanied by a cover letter, signed and dated by corresponding author specifying the following statement: "The manuscript has been seen and approved by all authors and is not under active consideration for publication, has not been accepted for publication, nor has it been published, in full or part (except in abstract form). I hereby assign all copyright in the enclosed manuscript to Yakhteh Medical Journal". Corresponding author can also propose three peer reviewers in the field of their article.

***B. Copyright*:** All listed authors must sign the "copyright transfer agreement". In doing so, each author takes public responsibility for the article's content. No article will be published without a copyright transfer agreement signed by all the listed authors.

***C. Manuscript** preparation:* Manuscript should be prepared in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". Manuscript must be typed in a font size of at least 12 points, double spaced with margins of at least 2.5 cm (1 inch). Each of the following manuscript components should begin on a new page in the following sequence:

***Page1*:** Title page; providing the full title of the research, full name(s) of author(s), highest awarded academic degree, institutional affiliation(s), full postal addresses, telephone, fax and e-mail of corresponding author *both in Persian and English.*

***Page 2*:** Running title; a maximum of 5 words (40 spaces and letters).

***Page 3*:** Abstract and keywords. Abstracts (*both in Persian and English)* should be followed by the title (no author's name) in structural format of not more than 250 words and should state the *Objective, Materials and Methods, Results and Conclusion* .

Keywords: Up to five key words must be supplied by the authors at the foot of the abstract chosen from the Medical Subject Heading (*MeSH*). The keywords, together with author's name are used for online searches. They should therefore be specific and relevant to the paper.

The following components should be identified after the abstract:

Introduction

Materials and Methods

Sufficient details to enable experiments to be repeated, including source, company names and addresses, must be supplied. Note the requirement for ethical approval from all studies involving humans and animals. Procedures and types of study and analyses must be clearly specified.

Results

Discussion

Conclusion

Acknowledgements: This optional part should include a statement thanking those who assisted substantially

with work relevant to the study. A permission letter may be needed from those who have been acknowledged. Grant support should be included in this section.

Conflict of Interest: All conflict of interest (financial or otherwise) and all sources of financial support must be listed on the title page. These include providers of supplies and services from a commercial organization. All commercial affiliations must be disclosed, regardless of whether they provide the funding.

References: The references must be written based on the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, the Vancouver style. Thus the references are cited numerically in the text and listed in the bibliography by the order of their appearance. The Uniform Requirements style is based largely on an ANSI standard style adapted by the National Library of Medicine (NLM) for its databases; **http://www.icmje.org**. For example, to state a book, the arrangement of information must be based on the following order:

Author's name, Title of the book, Place of the publication, Publisher, Date of publication

Tables and Legends: Tables should be on separate sheets. All tables should be keyed into the text. A short descriptive heading should be given above each table and any footnotes and explanations underneath.

Figures: Figures must be numbered and keyed into the text. They must be sent in TIFF or JPG format with 300 dpi resolutions.

Abbreviations should be defined where first mentioned in the text. Please use the international and standard abbreviations and symbols .Standard units of measurements and chemical symbols of elements need not be defined.

D. Proofs: Authors are sent page proofs for checking the production editor after acceptance of the article. Proofs are sent by e-mail as PDF files and should be checked and returned within 48 hours of receipt. It is the ***authors' responsibility*** to check that all the text and data as contained in the page proofs are correct and suitable for publication. We request that authors pay particular attention to author names and affiliations as it is essential that these details are accurate when the article is published.

3. General information:

A. It is recommended to supply us with your article on the compact disk (CD). You can send your article by e-mail to **yakhteh@royaninstitute.org** or online submission which is available at this website: **www.yakhteh.org**. If the article is not prepared according to the format of Yakhteh Medical Journal, it will be rejected. Please send us one copy of typed and three Xeroxed copies of your article.

B. The order of article appearance in the Journal is not concerning the scientific characters of the authors.

C. Yakhteh Medical Journal has authority to accept or reject the articles.

***D*. The received articles will be submitted to at least 5 reviewers. If three reviewers pass their judgments on the article, it will be presented to the Editorial Board of Yakhteh Medical Journal. If Editorial Board have a positive judgment about the article, reviewers' comments will be presented to the corresponding author (the identification of the reviewers will not be revealed).** The journal will contact the corresponding author directly within 7-8 weeks. If authors do not hear from journal office after the time specified, please fax or email to journal's office. Executive manager will respond promptly to authors' message.

E. The authors of the articles may publish their articles in other journals provided only with the permission of the Editor-in-Chief.

F. Three reprints of the article will be supplied for the corresponding author free of charge.

IN THE NAME OF GOD

Dr. Saeed Kazemi Ashtiani

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.

PUBLISHED & SPONSORED BY:
Royan Institute (ACECR), P.O.BOX: 19395-4644, Tehran, Iran

Indexed in:
1. Thomson Reuters (ISI)
2. Index Medicus for the WHO Eastern Mediterranean Region (IMEMR)
3. Index Copernicus Division
4. Cambridge Scientific Abstract (CSA)
5. EMBASE
6. Scopus
7. Magiran
8. Scientific Information Database (SID)
9. Iranmedex
10. Regional Information Center for Sciences and Technology

Abstracts of Royan International Twin Congress
9th Congress on Reproductive Biomedicine 4th Congress on Stem Cell Biology & Technology

Contents:

Editorial Office Address (Dr. Ahmad Hosseini):
Royan Institute, P.O.Box: 19395-4644, Tehran, Iran
Tel & Fax: (+9821)22510895
www.yakhteh.org, www.SID.ir
Email:info@yakhteh.org
yakhteh@royaninstitute.org

Yakhteh

Scientific Board

Collaborator

-Ahwaz Fertility Center
-Akbarabadi Hospital, Shahid Beheshti University of Medical Sciences
-Anatomy Department, Medical Sciences Faculty of Tarbiat Modares University
-Avesina Fertility Institute
-BMT Research Center of Shariati Hospital, Tehran University of Medical Sciences
-Department of Epidemiology and Biostatistics, Public Health School
-FMF Medical Imaging Center
-Hamadan Fatemiyeh Fertility Center
-Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences
-Infertility Department, Shariati Hospital
-Isfahan Fertility Center
-Mahdieh Hospital, Shahid Beheshti University of Medical Sciences
-Mehr Infertility Institute of Rasht
-Mirzakoucakkhan Hospital Fertility Center
-Mostafa Khomeini Hospital, Shahed University of Medical Sciences
-National Institute for Genetic Engineering and Biotechnology, Special Medical Center
-Omid Fertility Center
-Rasht Family IVF Center
-Sarem Fertility Center
-Shiraz Infertility Research Center
-Shiraz University of Medical Sciences
-Sina Hospital, Tehran University of Medical Sciences
-Tabriz Al-Zahra Hospital Fertility Center
-Urology Research Center, Tehran University of Medical Sciences
-Vali-e-Asr Reproductive Health Research Center (V.R.H.R.C.), Tehran University of Medical Sciences
-Yazd Infertility Research & Clinical Centre
-Zeinabyie Fertility Center, Shiraz University of Medical Sciences

Congress Chairman

Abdolhossein Shahverdi, Ph.D.

It is my honor and privilege to welcome you to the Royan International Twin Congress, which is the 9th Congress on Reproductive Biomedicine and the 4th Congress on Stem Cell Biology and Technology.

The aim of this congress is to bring a friendly atmosphere for scientists to exchange their knowledge and experiences covering the newest issues in infertility, assisted reproduction techniques and reproductive health plus newest advancements in stem cell biology, differentiation, cell therapy and tissue engineering.

On behalf of the organizing committee, I would like to extend our appreciation and thanks to the invited chairpersons, speakers and delegates for their great support and contribution making this event successful.

This year we have received several high level scientific abstract making the jury procedure more difficult than before. We took benefit from our scientific board containing many prominent professors from all around the world who were highly expert in their field and reviewed and evaluated all the abstracts and chose the best for the presentation.

Here by, I thank you one and all for coming to this congress and giving the privilege of sharing your knowledge and wisdom.

I am thankful to all members of the organizing committee and scientific board for their continuous efforts and indefatigable endeavor making this scientific event a great success.

Abdolhossein Shahverdi

Congress Honorary Chairman

Safaa Al-Hasani, D.V.M, Ph.D.

First of all, I would like to express my cordial thank to the Royan Institute for inviting me as Honorary Chairman of the 9th Royan International Congress on Reproductive Biomedicine and 4th Royan International Congress on Stem Cells Biotechnology.
On behalf of Dr. Abdolhossein Shahverdi and the organising committee of the Royan Institute I would like to welcome the invited speakers and the participants of this congress and to thank all of you for coming from all over the world to the great city of Teheran to join in this congress and so making it an excellent forum for exchanging knowledge in both Reproductive Medicine and Stem Cell Research. These two fields complement each other well and I am looking forward to fruitful discussions. To see so many people from so many different countries fills me with pride. It is an evidence for the importance of such meetings as so many scientists and researchers have followed the invitation. At the same time this demonstrates the role of the Royan Institute which has gained a lot of reputation. I have a special relation to the Royan Institute since I have been the first trainer of the department of Assisted Reproduction Technology (ART) and this makes me even more proud of the fact that the Institute has become one of the most famous and successful Reproductive Medicine and Research Centers in the Middle East for treating childless couples. At the same time, the Royan Institute is very active in the field of stem cell research as many excellent scientists are working on a variety of stem cell types.
I am sure this congress will be a great opportunity for all participants to share their results, to hear about each others experiences, and hopefully everyone will benefit from one another. Last but not least I wish you all a pleasant stay here in Teheran and hope that you will enjoy the congress as well as the social programme.

Safaa Al-Hasani

Andrology

I-1: Efficacy of Antioxidant Treatment in Male/Female Infertility: Evidence Based Medicine

Agarwal A

Departments of Obstetrics/ Gynecology, Anatomic Pathology, and Immunology, Cleveland Clinic Foundation, Cleveland, Ohio, USA
Email: Agarwaa@ccf.org

Oxidative stress has a significant impact on male and female fertility and ART outcomes. Effectiveness of antioxidant therapy depends on the cause of infertility. Therapeutics directed against each specific etiology of elevated ROS should be attempted first. Once the primary cause of infertility has been treated or no specific etiology can be identified (idiopathic infertil¬ity), patients may be advised to take optimal antioxidant supplementation doses. A variety of clinical trials have reported the beneficial effects of antioxidants in selected cases of male infertility, whereas others have failed to report similar ben¬efits. Pregnancy, the most relevant outcome param¬eter of fertility, was reported in only a few published stud¬ies. Some of the problems in assessing the efficacy of anti¬oxidants in men with infertility is the ability of spontaneous improvement in semen quality and pregnancy being dependent on female fertility status. The lack of consensus regarding the use of antioxidants in infertile men is because of insufficient studies and heterogeneous methods used in the studies conducted. Patient selection must also be considered in the forthcoming studies since oxidative stress may not be the only cause of male infer¬tility. There are only a few reports of antioxidant thereapy in female infertility; these treatments have not been successful in improvement of infertility conditons. Treatment strategies using antioxidant supplementation to reduce OS need additional investigation via randomized controlled trials.

I-2: Sperm DNA Damage Testing in Assisted Reproduction: Evidence Based Medicine

Agarwal A.

Departments of Obstetrics/ Gynecology, Anatomic Pathology, and Immunology, Cleveland Clinic Foundation, Cleveland, Ohio, USA
Email: Agarwaa@ccf.org

Pathologically increased sperm DNA fragmentation is one of the causes of repeated assisted reproduction failures in the ICSI era. Several studies have demonstrated that sperm DNA integrity correlates with pregnancy outcome in in vitro fertilization. Therefore, sperm DNA fragmentation should be included in the evaluation of the infertile male. Assessment of sperm DNA damage appears to be a potential tool for evaluating semen samples prior to their use in assisted reproduction. It allows the selection of spermatozoa with intact DNA or with the least amount of DNA damage for use in assisted conception. It provides better diagnostic and prognostic capabilities than standard sperm parameters for male fertility potential. The assessment of sperm for chromatin abnormalities may serve to provide a definitive diagnosis of the underlying causes of what thus far has been labeled as "idiopathic" and "unexplained infertility". This may also identify the group of men who, through techniques such as intracytoplasmic sperm injection, may perpetually propagate their genetic complement that is linked to male infertility.

I-3: Best Infertility Treatment for a Man with Varicocele: Assisted Reproduction or Varicocelectomy?

Pasqualotto FF

University of caxias do Sul,Urology Department,Caxias do sul, Brazil.
Email: fabio@conception-rs.com.br

Varicoceles are present in 15 percent of the normal male population and in approximately 40 percent of men presenting with infertility. The preponderance of experimental data from clinical and animal models demonstrates a deleterious effect of varicoceles on spermatogenesis. Testicular temperature elevation and oxidative stress appear to play an important role in varicocele-induced testicular dysfunction, although the exact pathophysiology is not yet completely understood. The American Urological Association and American Society of Reproductive Medicine jointly convened Best Policy Practice Groups for Male Infertility and recently stated, "Varicocele repairs may be considered the primary treatment option when a man with a varicocele has suboptimal semen quality and a normal female partner." They considered percutaneous embolization and surgery for varicocele treatment, and noted that most experts performed inguinal or subinguinal microsurgical repairs to maximize preservation of arterial and lymphatic vessels while reducing the chances of persistence or recurrence. Although these comments represent the considered opinion of 12 experts and 125 consultants in the field of male infertility, anyone familiar with varicoceles knows that discussion of the pathophysiology and management continues to be hotly debated. This review offers recommendations regarding the best infertility treatment for the man with a varicocele: assisted reproduction or varicocelectomy.

I-4: Best Infertility Treatment for the Vasectomized Men: Assisted Reproduction or Vasectomy Reversal?

Pasqualotto FF
University of caxias do Sul,Urology Department,Caxias do sul, Brazil
Email: fabio@conception-rs.com.br

In men with prior vasectomy, microsurgical reconstruction of the reproductive tract is more cost-effective than sperm retrieval with in vitro fertilization and Intracytoplasmic sperm injection if the obstructive interval is less than 15 years and no female fertility risk factors are present. If epididymal obstruction is detected or advanced female age is present, the decision to use either microsurgical reconstruction or sperm retrieval with in vitro fertilization and intracytoplasmic sperm injection should be individualized.
Sperm retrieval with in vitro fertilization and Intracytoplasmic sperm injection is preferred to surgical treatment when female factors requiring in vitro fertilization are present or when the chance for success with sperm retrieval and Intracytoplasmic sperm injection exceeds the chance for success with surgical treatment.

I-5: Testicular Apoptosis in Infertile Men with Varicocele and the Relationship with HPV Infection

Pasqualotto FF*, Fiedler G, Cezar TU, Pasqualotto EB

University of Caxias do Sul,Urology Department, Caxias do Sul, Brazil.
Email: fabio@conception-rs.com.br

Objective: Infertility affects 15% of couples around the world. Male factor accounts for 40-50% of the cases and varicocele is the most frequent cause of male infertility. However, the mechanisms involved in the pathophysiology of this disease are still not well elucidated. Cellular apoptosis is raised in infertile patients and research on apoptosis markers may provide new insights on the pathogenic mechanisms involved. We assessed testicular biopsies of men undergoing varicocelectomy and men undergoing vasectomy reversal to serve as a control, and evaluated apoptosis markers and the presence of HPV-DNA through PCR.
Materials and Methods: Testicular biopsies from 44 men (33 men undergoing varicocelectomy and 11 undergoing vasectomy reversal) who served as a control group. In patients with varicocele, testicular biopsy was performed bilaterally in 30 patients and unilaterally in 3 patients. In the 11 patients undergoing vasectomy reversal, testicular biopsy was performed bilaterally in 8 and unilaterally in 3. The apoptotic markers evaluated were M30, Bax, Bcl-2 by immunohistochemistry and the presence of HPV-DNA through PCR.
Results: We could not find, in our study, any significant difference in the apoptotic markers (M30, Bax, Bcl-2) between the varicocelectomy patients and the control group ($p>0.05$). HPV-DNA type 16 was found in 43.2% of the samples and, once again, a relationship between the presence of the HBP-DNA and the apoptotic markers was not detected ($p>0.05$).
Conclusion: According to our results, there is no relation between male infertility caused by varicocele and increased rates of apoptosis or HPV infection

Keywords: Testicle, Varicocele, HPV, Apoptosis

Embryology

I-6: Cancellation of Fresh Embryo Transfer: Future Perspective

Al-Hasani S.

Department of Gynecology and Obstetrics, University of Schleswig-Holstein, Campus Lübeck, Germany
Email: sf_alhasani@hotmail.com

Ovarian hyperstimulation syndrome (OHSS) and multiple births are the two major complications associated with in vitro fertilization (IVF). The incidence of OHSS has been reported to be as high as 33%, with severe OHSS occurring in 0.5–4% of patients. Generally, OHSS is preceded by multiple follicular developments combined with a high serum estradiol concentration. Luteinization is essential for its development.
Since the etiology of OHSS remains unknown and the pathophysiology is poorly understood, it is not surprising that no strategy has yet been shown to completely prevent the occurrence of severe OHSS, short of canceling the cycle. Many preventive methods have been evaluated including early ovarian puncture, glucocorticoids, intravenous albumin, and the prolonged use of gonadotrophin-releasing hormone agonist (GnRHa). No method has consistently demonstrated superiority in prevention of this syndrome. Triggering ovulation in a GnRH-antagonist protocol using gonadotrophin-releasing hormone (GnRH) agonists instead of human chorionic gonadotrophin (HCG) was first introduced by Itskovitz et al (1988,1991), and has been used ever since with excellent results in terms of OHSS prevention.
One approach which minimizes HCG exposure without forfeiting oocyte retrieval is the elective cryopreservation of all resulting pre-embryos, subsequently avoiding further HCG exposure during the cycle at risk. Vitrification of human oocytes and embryos became a more popular alternative to the slow rate freezing method due to reported comparable clinical and laboratory outcomes. In addition to recent publications which have suggested that GnRH agonist trigger may lead to significantly impaired implantation and ongoing pregnancy rates in fresh embryo transfer (ET) cycles in normal responders, as well as in high responders.
Based on this approach, we adopted a strategy where, final oocyte maturation with GnRH agonist is done, followed by elective vitrification of all two pronucleate (2 PN) oocytes and transfer of embryos in subsequent frozen–thawed embryo transfer cycle(s) (FT-ETs).
Keywords: Ovulation, Vitrification.
Materials and Methods: In-vitro fertilization patients ($n = 65$) enrolled in this prospective study at the IVF University Unit, between December 2004 to August 2007

were identified as being at high risk of OHSS (based on (i) ≥20 follicles ≥10 mm or E2 ≥ 4000 pg/ml at the time of induction of final oocyte maturation, (ii) history of cycle cancellation due to OHSS risk, (iii) the development of severe OHSS in a previous cycle). After Ovarian stimulation with GnRH-antagonist administration, final oocyte maturation was triggered by 0.2 mg triptorelin subcutaneously (s.c). All two pronucleate (2 PN) oocytes were cryopreserved by vitrification, and frozen–thawed ETs (FT-ETs) were performed in programmed cycles using exogenous estrogen and progesterone for endometrial preparation. All patients were given warnings for severe OHSS and were followed carefully for up to two week's post-retrieval.

Results: Sixty five patients were triggered with GnRH agonist, and underwent FT-ETs. To date, 265 vitrified zygotes have been warmed. The post thaw survival rate was 97 % (258/ 265). The mean embryos transferred per cycle were 2.3. The clinical pregnancy rate was 46.2% (30/65) per patient and 27.8 %(30/108) per thawed cycle. Up to date we have 13 deliveries (3 twin pregnancy) and no cases of moderate or severe OHSS were observed.

Conclusion: The GnRH agonist triggering for the final oocyte maturation after GnRH antagonist co-treatment combined with elective vitification of 2 PN reduces the risk of moderate and severe OHSS in high-risk patients undergoing IVF/ICSI without affecting pregnancy rate. We conclude from our study that adequate stimulation in good responders with sufficient zygotes for vitrification, save the patients from further oocyte pick up.

I-7: Vitrification of Human Zygotes and Embryos

Al-Hasani S.

University of Schleswig-Holstein, Department of Gynecology and Obstetrics, Section of Andrology, Campus of Luebeck, Ratzeburger Allee 160, 23560 Luebeck-Germany
Email: sf_alhasani@hotmail.com

Introduction: Slow-cooling (SC) cryopreservation of supernumary pronuclear stage oocytes during IVF/ICSI is well established and routinely implemented in the clinical IVF-programme. Recently, worldwide high survival and pregnancy rates with Cryo-Embryotransfer by vitrification using minimum volume cooling method have been reported. The radical strategy of vitrification is to result in a total elimination of ice crystal formation, both within the cells being vitrified (intracellular) and the surrounding solution (extracellular). In the present study, we examined the survival rate of vitrified and rewarmed human pronuclear stage oocytes that were cultured for additional 24 h before Cryo-ET as well as to evaluate the pregnacy rate. The results were compared to survival- and pregnancy rate using the slow-cooling cryopreservation method retrospectively.

Materials and Methods: Between January 2000 and November 2005 a total of 752 patients had 3616 supernumary zygotes during IVF/ICSI treatment. These zygotes were cryopreserved using the slow-cooling method. A total of 1005 supernumary zygotes from 211 other patients were vitrified between April 2004 and January 2008 using the Cryotop (Kuwayama, RBM-online, 2005, pp 608-615). For vitrification, zygotes were placed into equilibration solution (7.5% Ethylenglycol; 7,5% DMSO) and incubated for 8 min. at room temperature (RT). Hereafter zygotes were incubated in vitrification solution (15% Ethylenglycol; 15% DMSO; 0,5M Saccharose) for 45-60 sec. at RT and placed on the Cryotop-strip and were plunged directly into the liquid nitrogen. After Vitrifiction a hard plastic cover is attached to protect the strip during storage in liquid nitrogen. In total 1438 zygotes were thawed according to the conventional Slow-cooling-protocol. 107 zygotes were rewarmed after being vitrified: the hard plastic cover was removed in liquid nitrogen and the Cryotop was plunged in thawing solution (1M Saccharose) at 37 C for 1 min. Zygotes were placed in diluent solution (0,5M and 0.25M Saccharose) at RT each for 3 min. Washing was done many times before culture. After both procedures, vitality of zygotes was evaluated under dissecting microscope one hour after rewarming. Embryo transfer was done 24 hours after culture in programed cycles. Clinical pregancies per Cryo-ET were evaluated and compared for both methods.

Results: In total 1438 zygotes were thawed after being cryopreserved with the slow-cooling method. 848 zygotes seemed to be vital after thawing with a survival rate of 59%, while 381 zygotes were rewarmed after being vitrified corresponding to a survival rate of 96.3%.
583 patients underwent Cryo-ET after Slow-cooling procedure of zygotes. The clinical pregnacy rate per Cryo-ET was 10.2% (n=111). In contrast 115 patients underwent Cryo-ET after vitrification of zygotes. Pregnacy rate was 33.3% (n=69). Out of these 39 healthy babies were born.

Conclusion: These retrospective comparative results clearly demonestrate, that the Cryotop vitrification method of supernumary zygotes showed a high post-thaw survival and pregnancy rates suggesting that the Vitrification-protocol may be preferable because of its simplicity, cost-effectiveness and time saving in a busy laboratory daily-work.

I-8: Morphology of Fresh and Vitrified Blastocysts

Ebner T.

Landes- Frauen- und Kinderklinik, IVF-Unit, Krankenhausstr. 26-30, A-4020 Linz, Austria
Email: thomas.ebner@gespag.at

Though in slow freezing of blastocysts some post-thaw morphological predictors of pregnancy have been investigated, no such data have been published for vitrified and warmed blastocysts. Therefore, a prospective four-part score was applied to vitrified/warmed blastocysts to evaluate if certain morphological parameters could

act as predictors of implantation, pregnancy, and life birth. All blastocysts considered to be viable after warming were scored according to a previously unpublished grading system based on re-expansion, hatching (out of artificial gap in the zona pellucida), extensive cytoplasmic granulation and presence of necrotic foci. Overall, 74% of the vitrified blastocysts were found to be viable after warming. Early blastocysts showed better survival as compared to extended/hatching blastocysts ($p<0.01$). Out of the morphological parameteres analyzed, immediate re-expansion ($p<0.05$) and spontaneous hatching ($p<0.001$) were positive predictors of the rates of implantation, pregnancy and life birth. The opposite hold for exensive cytoplasmic granulation ($p<0.05$) which was negatively correlated. Accurate scoring of warmed blastocysts (within the first two hours) allows for prediction of pregnancy outcome and, thus, will help to further reduce the number of transferred embryos.

I-9: The Importance of Oocyte Quality in IVF

Ebner T.

Landes- Frauen- und Kinderklinik, IVF-Unit, Krankenhausstr. 26-30, A-4020 Linz, Austria
Email: thomas.ebner@gespag.at

MII-stage oocytes collected from patients following controlled ovarian hyperstimulation show varying qualities. Both nuclear and cytoplasmic maturation have to be completed in a coordinated mode to ensure optimal conditions for subsequent fertilization. Disturbances or asynchrony of these processes may result in different morphological abnormalities depending on whether nuclear or cytoplasmic maturation has been affected. With respect to this, it has been suggested that dysmorphic features that occur early in meiotic maturation could be associated with a higher frequency of aneuploidy and fertilization failure, while those occurring late in maturation may cause a higher incidence of developmental failure. In fact, more than half of the gametes collected show morphological abnormalities, some of which seem to be correlated with an impaired outcome (e.g. aggregation of endoplasmic reticulum, vacuolization, increased ooplasm viscosity, giant eggs). Therefore, it is strongly recommended to include oocyte quality in all scoring systems applied in IVF laboratories.

I-10: DNA Structure, Fragmentation and their Effect on IVF/ICSI Outcome

Hammadeh ME*, Fischer-Hammadeh C, Schmidt W.

Department of Obstetrics & Gynaecology, University of Saarland, Homburg/Saar, Germany
Email: mehammadeh@yahoo.de

DNA in the spermatozoa is a condensed, compact structure. Up to 85% of DNA is bound to protamine in complexes that are more compact than the DNA-histones complex in somatic cells (Erenpreiss et al., 2006). DNA in mammalian sperm is tightly compacted into linear arrays organized as loop domains (Ward & Coffey., 19991).

The integrity of DNA in the chromosomes of the spermatozoon is a prerequisite to normal fertilization and transmission of paternal genetic information (Agarwal and Said. 2003).

Poor semen quality has been associated with an increase in the proportion of sperm with DNA fragmentation (Lopes et al., 1998; Sun et al., 1997; Irvine et al., 2000).

It is clear that abnormalities in the male genome characterized by damaged DNA may be indicative of male sub fertility regardless of the routine semen parameters (Loops et al., 1998; Sakkas et al., 2000; Aitken & Krausz., 2001). Accumulating evidence suggest that disturbances in the organization of genomic material in sperm nuclei are negatively correlated with the fertility potential of the spermatozoa. DNA fragmentation is characterized by single-and double–strand DNA breaks, which are often detected in the ejaculates of subfertile men (Irvine et al., 2000). While many studies have established that DNA damage is present in sperm, the biological significance of these damaged cells in reproduction remain unclear. The utility of sperm DNA damage as a diagnostic and prognostic tool in the human fertility clinic has been extensively studies by many authors (Evenson et al., 1999)

The extent of DNA damage is closely related to sperm function and male infertility (Sakkas et al., 1999; Aitken, et al., 1999). A significant negative correlation was seen in IVF samples between the percentage of sperm with DNA damage and the fertilization rate and embryo cleavage rate ($p=0.008$; $p=0.01$ respectively) (Sun et al., 1997).

However, no association was seen between spermatozoa with DNA strand breaks and fertilization rate in ICSI patients (Host et al., 2000). It is of interest to mention that other studies found a significant negative association between the percentage of sperm with DNA fragmentation and the fertilization rate following ICSI ($r=-0.23$; $p=0.017$). In another study, the quality of embryos obtained, embryo development and the rate of ongoing pregnancies in relation to DNA fragmentation examined by TUNEL assay was examined in a selected group of 50 patients undergoing IVF and 54 ICSI. The authors found that DNA fragmentation did not influence the fertilization rate and embryo development in either IVF or ICSI when the DNA fragmentation rate was less than 10%. (Benchaib et al., 2003).

The influence of chromatin integrity on post embryonic development is the subject of intense investigation. There is a wider agreement concerning their negative effect on embryo development and pregnancy rate (Ahmadi & NG., 1999; Morris et al., 2002; Host et al., 2000; Larson et al., 2000). Reports indicate that the damage to sperm DNA may be linked to an increase in early embryo death (Sakkas et al., 1999).

Therefore, the assessment of sperm DNA damage appears to be a potential tool for evaluating semen samples prior to their use in ART.
This lecture will identify the origin, cause and clinical significance of DNA damage in male infertility and highlights its relevance in ART.

I-11: Smoking and Infertility

Hammadeh ME*, Fischer-Hammadeh C, Schmidt W.

Department of Obstetrics & Gynaecology, University of Saarland, Homburg/Saar, Germany
Email: mehammadeh@yahoo.de

A number of comprehensive reviews of the literature have been published, summarizing the cumulative data supporting an association between cigarette smoking and diminished female fecundity. In addition, evidence linking cigarette smoking to diminished fertility in the male has been reviewed.
Active smoking was associated with failure to conceive within 6 or 12 months with increase delay correlated to increase number of cigarettes smoked per day. The percentage of women experiencing conception delay for over 12 months was 54% higher for smokers compared to nonsmokers. Exposure to passive smoke further increased the odds against a woman conceiving within 6 months. The investigators studied data from nearly 15,000 pregnancies.
Cooper et al. also found a diminished ovarian reserve based on markedly statistically significant higher mean basal FSH levels, particularly in younger women smokers. Smokers also required more gonadotropins for ovarian stimulation in vitro fertilization cycles, had a higher number of canceled cycles, fewer oocytes retrieved in those cycles not canceled, more cycles with failed fertilization and a lower overall clinical pregnancy rate. This is one of several studies documenting diminished success in Assisted Reproduction among smokers compared to non-smokers.
Zenzes documented cotinine, a major metabolite of nicotine, to be found in dose dependent concentrations relative to the number of cigarettes smoked in 100% of the follicular fluids of infertility patients undergoing in vitro fertilization oocyte retrieval. Besides, 84% of women reporting themselves as non-smokers and with a nonsmoking partner also had detectable levels of cotinine in their follicular fluids. Also, many nonsmokers are regularly exposed by inhalation of 'sidestream' smoke from burning cigarettes and/or from 'passive' smoke exhaled by smokers.
Smoking is clearly associated with an increase in spontaneous miscarriage, with bacterial vaginosis (which is associated with late pregnancy miscarriage), with preterm labor and with delivery of low birth weight infants at added risk of neonatal morbidity and mortality.
To the adverse effects of cigarette smoking on female fertility and pregnancy outcome must also be added the adverse effects of cigarette smoking by males on their own fertility and on their partners through passive and sidestream smoke Several studies over many years have evaluated the effect of smoking on semen parameters, especially density, motility and morphology. These studies collectively demonstrate a reduction in sperm density, motility and possibly morphology. The reduction in sperm count averaged 22%, and showed a dose response, with increased cigarette smoking correlating to a greater reduction in sperm count.
Women who smoke have decreased fertility. The risk of spontaneous abortion is higher for pregnant women who smoke. Babies born to smokers weigh, on average, 200 grams less than babies born to comparable women who do not smoke, with low birth weight being an important predictor of infant mortality.

I-12: Vitrification

Ingolf Nielsen H.

MediCult a/s, Møllehaven 12, DK-4040 Jyllinge, Denmark
Email: hin@medicult.com

Cryopreservation of reproductive entities is gaining a constantly growing importance. What used to be a means for storage of semen and embryos now has a much wider application. On top of being part of ongoing subfertility treatment, it now also plays an important role in extension and preservation of fertility.
We see an increase in cumulative pregnancy rate, a possibility of single embryo transfer, a less troublesome second treatment, a possibility to deal with legal restrictions (e.g. Italy), and a number of practical issues.
We have the possibility of extending and preserving fertility of health reasons, e.g. cancer and premature menopause, and of social and personal reasons varying from country to country.
With more or less success, we are now able to preserve all types of reproductive structures – from gonadal tissue and immature oocytes to hatched blastocysts. Still there are only two main methods – slow freezing and vitrification.
This lecture will touch on slow freezing, but mainly look at various aspects of vitrification: What is vitrification, which tools are used, how is it done, and how successful is it?

I-13: Laboratory Quality Management

Ingolf Nielsen H.

MediCult a/s, Møllehaven 12, DK-4040 Jyllinge, Denmark
Email: hin@medicult.com

The lecture will deal with Good Laboratory Practice and Laboratory Quality Control in general, but with special reference to IVF.
Common sense and responsibility remain extremely important, but there are now special guidelines published

by organisations like ESHRE, which can help ART units perform optimally.
A quality management program consists of Quality Control, Quality Assurance and Quality Improvement. It is important to have a written quality policy highlighting the necessity of integration between the individual departments and functions like gynecology and embryology. Standard Operation Procedures (SOP) must be written and adhered to.
Different aspects of laboratory QC, like biological specimens, patients, culture conditions, equipment, disposables etc., will be discussed. So will QA, like implantation rate, biochemical pregnancy rate, clinical pregnancy rate, baby-take-home rate, multiplet rate, survival rate after freeze-thaw etc.

I-14: The Molecular Basis of Implantation of the Human Embryo: Dlues from in Vitro Models

Mardon HJ.

Nuffield Department of Obstetrics and Gynaecology, Division of Medical Sciences, University of Oxford, Women's Centre, Level 3, John Radcliffe Hospital, Headington, Oxford, OX3 9DU
Email: helen.mardon@obs-gyn.ox.ac.uk

I-15: Ligand- receptor interactions in implantation of the human embryo

Mardon H. J.

Nuffield Department of Obstetrics and Gynaecology, Division of Medical Sciences, University of Oxford, Women's Centre, UK
Email: helen.mardon@obs-gyn.ox.ac.uk

I-16: Gryopreservation of Spermatogonial Stem Cells

Movahedin M.

Anatomu Department, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran
Email: Mansoure@modares.ac.ir

I-17: New Era in Sperm Selection Procedures for ICSI

Nasr Esfahani MH.

Isfahan Capmus of Royan Institute, Isfahan, Iran
Email: mh_nasr@med.mui.ac.ir

ICSI has become the routine method of choice for treatment of male infertility. Even male factor is the major concern during this procedure, but still much emphasis has been directed on number and quality of oocytes recovered and just the presence of sperm has been taken as granted. However, literature study on the quality sperm reveal that in male factors, the semen contain large number of protamine deficient sperm, high number of apoptotic sperm, fragmented DNA or and high number of immature sperm. In addition these factors may directly or indirectly effect fertilization and subsequent development. Therefore, along with different sperm function tests, different sperm selection procedure has been developed mainly based on sperm surface characteristics. These include: 1) Hyaluronic acid binding test base on presence of sperm surface receptors, 2) Zeta potential, based on the surface electrical charge, 3) Magnetic sperm sorting base on apoptotic markers present on the sperm surface. The consequence of these procedures on assisted reproductive techniques will be discussed during presentation.

I-18: Contrasting Stories: Increasing Cloning Efficiency in Cattle Vs. Mouse

Oback BJ.

AgResearch Ltd., Ruakura Research Centre, Reproductive Technologies, East Street, Private Bag 3123, Hamilton, New Zealand
Email: bjorn.oback@agresearch.co.nz

Despite more than a decade of research efforts, farm animal cloning by somatic cell nuclear transfer (SCNT) is still frustratingly inefficient. Inefficiency manifests itself at different levels which are currently not well integrated. At the molecular level, it leads to widespread genetic, epigenetic and transcriptional aberrations in cloned embryos. At the organismal level, these genome-wide abnormalities compromise development of cloned fetuses and offspring. Specific molecular defects need to be causally linked to specific cloned phenotypes, in order to design specific treatments to correct them. Cloning efficiency depends on the ability of the nuclear donor cell to be fully reprogrammed into an embryonic state and the ability of the enucleated recipient cell to carry out the reprogramming reactions. Choosing somatic stem cells as donors has not improved cloning efficiency, indicating that donor cell type may be less critical for cloning success. Different recipient cells, on the other hand, vary in their reprogramming ability. In bovine, using zygotes instead of oocytes has increased cloning success. A similar beneficial effect has not yet been demonstrated in mouse SCNT experiments. Other improvements in livestock cloning efficiency include better co-ordinating donor cell type with cell cycle stage, and increasing the amount of reprogramming factors through double cytoplast transfer. Both approaches have met only limited success in mouse cloning. On the other hand, strategies that have dramatically increased cloning efficiency in the mouse, such as aggregating SCNT embryos and treating them with histone deacetylase-inhibitors, have not yet shown significant effects in bovine. In the future, it will be important to demonstrate if these observations reflect technical differences in the cloning procedure or are due to species-specific differences in epigenetic reprogramming.

I-19: Similar Stories: Cloning from Stem Cells in Cattle and Mice

Oback BJ

AgResearch Ltd., Ruakura Research Centre, Reproductive Technologies, East Street, Private Bag 3123, Hamilton, New Zealand
Email: bjorn.oback@agresearch.co.nz

Cloning efficiency depends on the ability of the nuclear donor cell to be fully reprogrammed into an embryonic state and the ability of the enucleated recipient cell to carry out the reprogramming reactions. It has been postulated that reprogrammability of the somatic donor cell epigenome is influenced by its differentiation status. This hypothesis is mainly supported by three lines of evidence from comparative mouse cloning experiments using 1) progressively advanced blastomere donor nuclei from early cleavage stages, 2) ES cells and 3) terminally differentiated lymphocytes and neurons. These comparisons demonstrated that early blastomeres result in much higher cloning efficiency than somatic cells. However, they failed to conclusively determine whether differentiation status significantly affects cloning efficiency within somatic donor cell lineages. More recently, tissue-specific stem cells and progenitors from six different somatic lineages have been evaluated for their suitability as nuclear donor cells. In mouse, cells of divergent differentiation status isolated from the neuronal, hematopoietic, mesenchymal and skin epithelial lineages were compared. In farm animals, progenitors and stem cells from the skeletal muscle (bovine) and antlerogenic lineage (deer) were compared to their in vitro differentiated progeny, myotubes and adipocytes, respectively. None of these studies have found any significant evidence that adult stem cells result in higher cloning efficiency than differentiated cells from either the same or some unrelated lineage, indicating that donor cell type may not be critical for cloning success. It remains to be seen if the limited number of NT experiments reported was not sufficient to detect a hierarchical relation between cell differentiation, epigenomic status of somatic stem cells and cloning efficiency or if such a relation is not universally true.

I-20: Human Sperm DNA Instability

Ozmen B[1,2], Al-Hasani S[2] *

1. University of Ankara, Department of Gynecology and Obstetrics, Center of Artificial Reproduction, Campus of Cebeci, Mamak, Ankara- Turkey
2. University of Schleswig-Holstein, Department of Gynecology and Obstetrics, Section of Andrology, Campus of Luebeck, Luebeck-Germany
Email: sf_alhasani@hotmail.com

Functions of seminal vesicle, prostate and bilateral testes can be determined by parameters of sperm analysis. However traditional spermiogram and classical morphological assessment even based on strict morphological criteria sometimes fail in certain prediction of reproductive outcome of infertile men. Nevertheless sperm DNA might comprise many useful clinical parameters and predictors for reproductive outcome. Conversely to loose structure of chromatin in somatic cells, chromatin is tightly compacted in human sperm. Sperm DNA damage caused by factors such as reactive oxygen species, febrile disease, testicular hyperthermia, varicoceles, drugs, chemotherapy, radiation, genital tract infections, environmental causes, genetic deficiencies and hormonal factors. There are several tests such as TUNEL, COMET assays, SCSA verify sperm DNA damage by detecting DNA breaks and decreased ratio of protamine/histone or the susceptibility to denaturation. Increased sperm DNA damage has been shown to be related with poor reproductive outcome. Some of the studies indicated only affected fertilization rates whereas other reported both poor embryo quality, and lower ongoing pregnancy rates. More or less the studies met on some topics that till four cell stage embryonic genome is not activated and resulting in unaffected embryo morphology before this stage. As well today recovery of sperm DNA damage by oocyte after fertilization forming healthy embryonic genome up to several damage degrees has been claimed. Therefore in the future sperm DNA damage seemed to have a severe role in assisted reproduction especially in selection of treatment choice.

I-21: Embryo-Endometrial Interactions in Eetablishment of Pregnancy

Salamonsen LA.

Prince Henry's Institute of Medical Research, Melbourne, Victoria 3168, Australia
Email: Lois.Salamonsen@princehenrys.org

Implantation is generally considered to begin with initial apposition and attachment of the trophectoderm to the maternal endometrial epithelium and to be complete only once the developing conceptus is adequately supplied with a blood supply from the mother. However, even prior to attachment, there is a complex interactive and highly synchronized embryo-maternal dialogue. Establishment of pregnancy depends on these interactions and on molecular processes that occur even before attachment.
For ethical reasons, it is not possible to study in vivo the precise interactions between the human blastocyst and the maternal endometrium during the earliest stages of implantation. Implantation in women differs in a number of aspects from other mammals
because of key differences in the physiology of the reproductive tract between species and in the highly invasive capacity of human trophoblast. Therefore, although animal models can be used to identify molecules of importance for implantation in women, extrapolation to the human requires the judicious use of ex vivo and in

vitro techniques, including cell culture models. Among the molecules identified as critical for implantation in mice are cytokines, chemokines, proteases and calcium binding proteins. These mediators are found also to be maximally expressed during the 'window of receptivity' in the endometrium, specifically in the epithelium and/or the decidualizing stroma in women – some are secreted into the uterine lumen and some may be regulated by blastocyst factors. Dysregulated production of these key molecules is likely to contribute to infertility while manipulation of their expression or action could be utilized to enhance fertility.

I-22: Unravelling Causes for Implantation Failure: Protemic Approches

Salamonsen LA.

Prince Henry's Institute of Medical Research, Melbourne, Victoria 3168, AUSTRALIA
Email: Lois.Salamonsen@princehenrys.org

Implantation failure can result from a poor quality blastocyst/embryo or from inadequately prepared endometrium. While the need for synchronous development of the embryo and the endometrium was identified in animals several decades ago and its importance is clear from IVF outcomes in women, the key features of the blastocyst and the endometrium that are essential for successful implantation are still not well understood. Emerging technologies, the 'Omics' are now being applied to the assessment of embryos and to determine the factors contributing to endometrial receptivity.

While genomic studies have provided considerable information, the transcripts detected are not necessarily translated and most proteins undergo considerable post-translational modification. Proteomics therefore offers certain advantages in a global approach. In terms of understanding endometrial receptivity, differential in gel electrophoresis (DiGE) coupled with mass spectrometry (MS) is providing important information.

To define key differences at the protein level, between proliferative and secretory phase endometrium, DiGE analysis of endometrium from the mid-proliferative vs the mid-secretory phase of the menstrual cycle was performed and detected 196 differentially expressed spots. Thirty one of the differentially expressed proteins were identified using MALDI-TOF MS. These grouped into seven categories, which included structural (7), transport (4), regulatory (2), membrane (2), enzyme (2), motor (1) and others (2). The top network for secretory endometrium clustered around TGF-β. Proteins released from the endometrium into the uterine lumen are likely to be important for blastocyst development. Analysis of uterine lavages taken in the mid-secretory phase revealed an abundance of serum proteins. Following selective removal of the 6 most abundant proteins, over 1050 spots were detected in the lavages. A proteomic approach is also being applied to endometrial samples taken at ovum pickup during IVF procedures. Principal components analysis following DiGE analysis of samples from three groups of women; fertile egg donors, those who achieved pregnancy in the cycle sampled, and women who were never pregnant (>5 IVF cycles), demonstrated that the expression profiles segregated in clusters as hypothesized. Thus, proteomic approaches are providing important new leads towards understanding endometrial receptivity and may provide clinically useful biomarkers.

Epidemiology and Ethics

I-23: Epidemiological Research in Reproduction Field

Kamali K.

Department of Epidemiology and Biostatistics, Public Health School, Tehran, Iran
Email: kkamali71@yahoo.com

Epidemiology is the study of how disease is distributed in populations and the factors that influence or determine this distribution. Reproductive epidemiology focuses a wide range of topics, from the development of reproductive systems to conception and pregnancy, delivery and health of the offspring, to reproductive senescence. The epidemiologic study of reproduction is complicated by some important methodologic problems that are not often seen in other areas of epidemiology. There are many endpoints of interest to the reproductive epidemiologist which are not independent and may compete directly with one another.

Because reproductive outcomes (e.g., pregnancy complications or spontaneous abortion) are relatively common, are incompletely ascertained, and often have a short time between exposure and effect, the usual advantages of case-control studies are less applicable for reproductive epidemiology, and cohort studies play a relatively more important role. The availability of repeated outcomes for a woman or couple (or a family over multiple generations) offers research opportunities that are not seen in many other areas of epidemiology. There are specific reproductive endpoints: Puberty and menopause, The menstrual cycle, Fertility, Pregnancy loss, Pregnancy complications, Birth weight and Birth defects. Special topics in reproductive epidemiology are: Semen quality, Time-to-pregnancy studies, Pregnancy loss, Perinatal mortality, Birth-weight or Gestational-age-specific mortality, Birth defects, Genetic factors, Reproductive epidemiology & Infectious disease, Psychosocial Aspects, Behavioral science, Molecular and Genetic applications in reproductive epidemiology.

I-24: Human Dignity, Payment and Contracts in Assisted Reproductive Techniques Using Third Party

Omani Samani R*, Rezania Moalem MR

Department of Epidemiology and Ethics, Royan Institute, Tehran, Iran
Email: samani@royaninstitute.org

The word 'donation' implies no payment and informed consent for donation is essential in respecting the autonomy of the giving party, but as is practiced in many centers in the world, there is a payment for the donor named "compensation" for the donor's time and also medication and anesthesia, … that the donor must take during the procedure. Need for a donor plus the payment brings a lot of ethical, moral and social problems that must be solved between donor, recipient and the institute. Need for a contract and informed consent along with detail counseling with both the donor and the recipients seems to be necessary. Since the contract subject is a part of human body (gamete), it is not easy to make a legal contract, because human body cannot be considered as a property. Religious issues interfere with any contract between people especially in a religious context like Iran. Other issues in donation like anonymity also make any contract more difficult. Here in this paper, the issues regarding contract in donation and controversies regarding human dignity, Islamic issues, social problems and some solutions are presented.

Keywords: Donation, ART, Gamete, Contract, Ethical, Social, Contract, Islam

I-25: Infertility Counselling Alleviating the Emotional Burden and Ensuring Appropriate Medical Service

Thorn P.

Langener Str. 37, 64546 Mörfelden, Germany
Email: mail@pthorn.de

Infertility is not only a medical condition; the inability to have a child has emotional and social implications. Infertility can result in emotional suffering and is often described as a severe life crisis. In the last decades, a shift has occurred from coping with or resolving infertility through social means (i.e. participating in rearing the children of others, adoption) to the availability of medical interventions – even when accessing them can be challenging because of a lack of resources. During the same period of time, infertility counselling has emerged as a specialist area within the field of psychology. From individual counsellors operating independently or in collaboration with clinics, this has evolved into the establishment of national professional organisations and in 2003 into the foundation of the International Infertility Counselling Organisation (www.iffs-iico.org).
This presentation will focus on psychosocial counselling in the area of infertility and reproductive medicine. It will provide an outline of international agreements on the relevant qualification of counsellors and tackle the issue of who should be counselled. In the last decades, a growing number of legislations have mandated clinics/doctors to recommend counselling to patients, esp. when gamete donation is used. This has given rise to the question as to what settings can be offered so that patients view counselling as a helpful opportunity to explore emotional reactions to and issues raised by infertility and its treatment. As a result, different forms of counselling and psychosocial care have been developed, including group work, educational workshops and the dissemination of written and spoken information on psychosocial aspects. A controversial issue is the question whether counsellors should carry out assessment, i.e. evaluate parental or donor suitability and whether these, under certain circumstances, should be excluded from treatment. Some countries have developed guidelines for assessment or passed legislation that regulates access to service, but in many, this is an unregulated and challenging issue.

I-26: Building a Family with the Assistance of ART Common Psychological Reactions to Infertility and its treatment

Thorn P.

Langener Str. 37, 64546 Mörfelden, Germany
Email: mail@pthorn.de

This presentation will center on the development of our understanding of the psychology of infertility. Original investigations into psychological aspects of infertility focused on individual psychopathology (such as unconscious rejection of motherhood), on sexual dysfunctions and infertility-specific stress. Research in the 1960ies and 1970ies was only emerging and often lacked scientific rigor not least because the taboo surrounding infertility hindered research.
In recent years, the focus has shifted from individual psychopathology to more holistic perspectives and of course to understanding the impact of assisted reproductive treatment (ART) on psychological and social well-being. Research indicates that infertile men and women do not experience significant levels of psychological trauma, but involuntary childlessness, the use of ART and esp. gamete donation result in a multitude of challenges that can considerably increase psychosocial distress. Thus, a dramatic shift had occurred from assuming psychological qualities to be the cause of infertility (psychogenic theories) to understanding that infertility per se and its treatment can cause psychological stress (psychological consequences theories). This approach, however, failed to consider social and cultural factors influencing the experience of infertility and its treatment.
Currently, a psychosocial context approach is discussed which recognises biopsychosocial factors as relevant for and impacting on individuals and couples. Under this premise, the individual suffering from infertility is seen as part of a social structure, i.e. a marriage, an extended

family, a social network and a specific culture and religion and this social structure is considered to impact on the individual' management and experience of infertility. This presentation will describe how-based on such a holistic understanding - different theoretical frameworks such as bereavement approaches, identity and stigma theory can be used to improve our understanding of psychological reactions to infertility while not ignoring the social and cultural context.

Female Infertility

I-27: Ovarian Hyper Stimulation Syndrome

Aflatounian A.

Research & Clinical Center for Fertility, Shahid Sadughi University of Medical Science, Yazd, Iran
Email: abbas_aflatoonian@yahoo.com

I-28: Tree-Dimensional Ultrasound in Multiple Gestations

Akhlagh Pour Sh.

Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

I-29: Laparoscopic Microsurgery

Alborzi S.

Shiraz University of Medical Sciences, Shiraz, Iran
Email: alborzis@sums.ac.ir

I-30: -Post Traumatic Stress Disorder Causing Avoidance to Get Pregnant-Case Report and Discussion

Alizadegan Sh.

Department of Endocrinology and Female Infertility, Royan Institute, Tehran, Iran

I-31: Laparoscopic Operations (Video presentation)

Asefjah H.

Shiraz University of Medical Sciences, Shiraz, Iran
Email: hossein.asefjah@gmail.com

Laparoscopy is a closed & two dimentional operation,the result of operation depends on many factors,the surgeon,s skill is the most important factor,availability & variability of instruments are the second factor.I will introduce a new myoma dissector which does 3 functions at the same time ,it can cut, cauterize & dissect
simultaneosly which shortens operative time & decrease blood loss.Application of this instrument &intracorporeal suturing technique will be shown.
The next operation is Laparoscopic Strassman Metroplasty of bi cornuate uterus,The mean prevalence of uterine malformations in infertile women is approximately 3.5%, and in patients with recurrent pregnancy losses, it is approximately 13%. Bicornuateuteri form in 25%of these cases. This is a case presentation of 26 year old lady who has had 2 second trimister prenancy losses. Finally triple puncture laparoscopic hystrectomy using Ligasure & set of uterine manipulator with no need for ureteral dissection will be presented.

I-32: Recombinant HCG

Esmat Kh.

Merck Serono Commercial International, 14/1 Al Shatre Altasee , new Maadi , Cairo, Egypt
Email: khaled.esmat@merckserono.net

I-33: Cardiovascular Disease Risk Factors in Women with Polycystic Ovary Syndrome

Farimani M[1*], Gharakhani M[2]

1. Obstetrics and Gynecology Department, Hamadan University of Medical Science, Hamadan, Iran
2. Cardiology Department, Hamadan University of Medical Science, Hamadan, Iran
Email: dr_farimani@yahoo.com

CVD remains the leading cause of death in women; Women with PCOS have a number of reproductive and metabolic abnormalities.
Obesity and metabolic abnormalities are recognized risk factors for the development of ischemic heart disease in the general population, and these are also recognized features of PCOS. The questions are whether women with PCOS are at an increased risk of ischemic heart disease.
The aim of our study was to determine the prevalence of cardiovascular risk factors in PCOS women in reproductive age.
Materials and Methods: In prospective study 204 women in reproductive age (15-45) with oligomenorrhea or secondary amenorrhea and with clinical manifestation of hyperandrogenism (hirsutism and/or acne and/or androgenic alopecia)included .Non of the patients have thyroid dysfunction and hyper prolactinemia. Information about weight ,height ,BMI and BP were collected by history and physical examination , hormonal assay ovarian volume were measured in 3th of cycle and FBS, lipid profile, TG, measured after 14 hours starvation and then analyzed with 10th version of SPSS.
Results: Data showed that only 29/9% of patients had normal BMI, and other s suffered from overweight and obesity, 4.% had hypertension and 2% had FBS>126mg/dl.These risk factors increased with aging. Low HDL was seen in 71.6% of patients. Mean of HDL levels was 38/16mg/dl. Patients with higher ovarian volumes

Distribution criteria of metabolic abnormality in PCOD women

Men HDL	Mean TG	Mean cholesterol	Mean FBS	BP	BMI mean	BMI>25	num	Age
33/77	213/11	208/88	83/44	0	25/76	5	9	15-21
41/08	141/42	196/35	88/31	1	27/48	52	90	21-25
41/64	155/21	198/72	86/58	2	29/27	52	67	26-30
39/82	180/51	201/72	89/44	3	30/24	26	30	31-35
34/66	170/66	179/33	97/33	0	36/43	3	3	36-40
38	169/20	195/20	97	2	27/50	1	5	41-45

Distribution criteria of cardiovascular risk factors in PCOD women respect to ovarian volume

Ovarian volume	num	Over weight and obesity	FBS>126	Increased BP	Hyper cholestromia	Increased TG	Increased LDL	Decreased HDL
<8cm3	8	4(50%)	1(2.5%)	1(12.5%)	1(12.5%)	1(12.5%)	0	3(37.5%)
8-16cm3	168	114(67.8%)	3(1.8%)	7(4.2%)	62(36.9%)	31(18.5%)	37(22%)	120(71.4%)
>16cm3	28	25(89.3%)	0	0	20(71.4%)	8(28.5%)	8(28.5%)	23(82.1%)

had higher BMI, total cholesterol and LDL besides lower HDL.

Conclusion: The data showed that the prevalence of cardiovascular disease risk factors such as low HDL-cholesterol and obesity can not be ignored, especially these risk factors increased with aging

Keywords: Cardiovascular Risk Factors Metabolic, Polycystic Ovarian Ssyndrome, Ovarian Volume

I-34: Screening of down syndrome-

Fattahi F

FMF Medical Imaging Center, Tehran, Iran

I-35: Embolization of Myoma

Golestaneh SA.

Mostafa Khomeini Hospital, Shahed University of Medical Sciences, Tehran, Iran

I-36: Ovulation and Ovarian Cancer

Hillier SG*, Rae MT, Harlow CR, Fegan KS.

Centre for Reproductive Biology, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh, UK
Email: S.Hillier@ed.ac.uk

Epidemiological studies link ovulation and epithelial ovarian cancer, which frequently develops from the ovarian surface epithelium (OSE). Ovulation can be likened to an inflammatory reaction initiated by the mid-cycle luteinizing hormone surge. Each ovulation involves proteolytic breakdown of the follicle wall and ovarian surface to allow shedding of the oocyte. Serial injury and repair of the OSE thereby creates the potential for inflammation-associated genetic damage leading to neoplasia. Since ovulation is normally so regular and frequent, natural mechanisms must exist to localize and limit ovarian inflammation and minimize emergent disease. Our research suggests a role for cytokine up-regulation of HSD11B1 gene expression in this process. HSD11B1 encodes the steroidogenic enzyme 11βhydroxysteroid dehydrogenase type 1 (11βHSD1) that metabolizes substrate cortisone to anti-inflammatory cortisol. We find 11βHSD1 mRNA and enzymatic activity strongly up-regulated by the ovulation-associated cytokine interleukin-1α (IL-1α) in cultured human OSE cells. Moreover, cortisol enhances IL1α-induced up-regulation of 11βHSD1 mRNA, providing feed-forward amplification of local anti-inflammatory signalling in these cells. We therefore propose that cytokine activation of cortisol regeneration via 11βHSD1 can provide a mechanism for anti-inflammatory 'protection' of ovary during ovulation. In support of this concept, we find that IL1α up-regulates matrixmetalloproteinase (MMP) 9 (gelatinase B) activity in OSE cells and this is suppressed by glucocorticoid receptor-mediated cortisol action in vitro. Suppression of MMP9 gene expression through locally regenerated glucocorticoids has implications both for normal ovulation and ovarian cancer spread. Many ovarian cancer cell lines are unable to respond normally to IL-α in terms of 11βHSD1 mRNA. Likewise, many primary epithelial ovarian cancer cells show a deficient 11βHSD1 mRNA response to IL-1α relative to normal OSE. If this translates into deficient glucocorticoid suppression of MMP9 in primary ovarian cancer, this could be a mechanism of promoting metastatic tumour spread in vivo.

I-37: The biology of Ovarian Ageing

Hillier SG

Centre for Reproductive Biology, Queen's Medical Research Institute, University of Edinburgh, UK
Email: S.Hillier@ed.ac.uk

Ageing is associated with reduced fertility in women, partly due to the progressive loss of ovarian follicles throughout life but also to an associated reduction in oocyte quality. A distinction can be made between biological ovarian age (i.e. loss of follicles) and chronological ovarian age, which varies from person to person and is likely set by multiple environmental and genetic queues. Usually at around 38 years of age, several years before menstrual cycles cease, the initiation of follicular growth begins to accelerate, speeding up the loss of the residual follicular stock. This alteration is associated with a gradual increase in circulating plasma FSH levels. This monotropic FSH rise appears mainly due to reduced secretion of follicular growth and differentiation

factors related to transforming growth factor-β (TGFβ) that negatively affect pituitary FSH release. In particular the clinical application of assays for inhibin (INH) and Anti-Müllerian Hormone (AMH) and has helped clarify the roles of these substances in ovarian physiology and permitted their use as potential biomarkers of ovarian ageing. It seems that as the number of (immature) INH B and AMH secreting follicles declines with age, negative feedback regulation of pituitary FSH secretion is relaxed. Basal circulating FSH levels rise accordingly, promoting inappropriate maturation of granulosa cells in residual preantral (INH B-secreting) follicles containing eggs that have not completed their gonadotrophin-independent growth phase. Presumably due to the asynchronous maturation of the germinal and somatic components of such follicles, they eventually become atretic. As FSH levels continue to rise the process is amplified until, in the late perimenopause, oestradiol and INH A levels also fall and menstrual cycles cease. Thereby FSH orchestrates the termination of oogenesis and folliculogenesis in the human ovary. This lecture explores the endocrine and paracrine basis of ovarian ageing and explains how the signalling molecules involved can serve as biomarkers of ovarian responsiveness to gonadotrophin therapy.

I-38: Immunological Aspects of Recurrent Miscarriage

Jeddi Tehrani M

Avesina Infertility Clinic, Tehran, Iran
Email: Mahjed@Avicenna.ac.ir

I-39: Sonohysterography

Kalantary M

Mahdieh Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Mojgan_kalantary@yahoo.com

SonoHysteroGraphy is a valuable & simple , minimally invasive ultrasound procedure that may be used to evaluate the endometrium.
SHG augments the traditional TVS examination by distending the endometrial canal with saline , which allows each individual layer of endometrial lining to be evaluated separately. The single-layer evaluation make possible with SHG significantly improves detection & characterization of focal and diffuse endometrial processes over that of TVS alone
The technique involves placement of a catheter into the endometrial canal with subsequent instillation of sterile saline solution under ultrasound guidance.

I-40: Metformin and Polycystic Ovarian Syndrome

Karimzadeh MA
Yazd Infertility Center, Yazd, Iran

I-41: New Methods for COH

Khalaf Y

Assisted Conception Unit, Guy's and St Thomas' Hospital Foundation Trust, London, UK
Email: yakoub.khalaf@kcl.ac.uk

I-42: Recurrent Failure Implantation

Khalaf Y

Assisted Conception Unit, Guy's and St Thomas' Hospital Foundation Trust, London, UK
Email: yakoub.khalaf@kcl.ac.uk

I-43: First Trimester Fetal Anomalies

Larijani T

Akbarabadi Hospital, Iran University of Medical Sciences, Tehran, Iran

I-44: Nutrition and Polycystic Ovarian Syndrome

Madani T

Department of Endocrinology and Female Infertility; Royan Institute, Tehran, Iran
Email: tmadani@royaninstitute.org

Polycystic ovary syndrome is a common disorder in women of reproductive age. The estimated prevalence is 4-8%. PCOS is associated with a number of reproductive disorders and is characteristic by the presence of polycystic ovaries, menstrual dysfunctions, infertility or reduced fertility, biochemical or clinical hyper androgenism; increase the risk of cardio metabolic disturbances including dyslipiemia and diabetes.
Although the pathogens of PCOS is complex and not entirely understood, obesity (particularly abdominal obesity is mediated by the development of insulin resistance and is closely linked to the development of this condition and its clinical feature, particularly menstrual irregularities and increased androgen.
Most patients with PCOs are obese and insulin resistant. Life style modification focusing predominantly on diet and exercise behavior is considered the preferred first-line treatment for PCOs management.
Several studies have shown that weight loss of 5-10% of weight in PCOs patient via energy restriction can reduce circulating insulin level and hyperandrogenism. According to this idea we allocate so PCOs patients, who losing their weight and we compare the clinical and biochemical findings of them.

I-45: Comparison of Microflare with other Protocols in Poor Responders

Madani T

Department of Endocrinology and Female Infertility; Royan Institute, Tehran, Iran

Email: tmadani@royaninstitute.org

I-46: Current and New Medical Treatment of Endometriosis

Mehrafza M

Mehr Infertility Institute, Rasht Iran

Background: Endometriosis is a common gynaecological condition which affects many women of reproductive age worldwide and is a major cause of pain and infertility.
Objective: To evaluate the literature on the current and new medical treatments for endometriosis.
Materials and Methods: Review of Pubmed, Cochrane database and Medline for current review articles and studies regarding the usual and new medical treatment strategies for endometriosis.
Results: Endometriosis can be treated with medications and/or surgery. The goals of endometriosis treatment may include pain relief and/or enhancement of fertility.
Nonsteroidal anti-inflammatory drugs or NSAIDs are commonly prescribed to help relieve pelvic pain and menstrual cramping. Since endometriosis occurs during the reproductive years, many of the available medical treatments for endometriosis rely on interruption of the normal cyclical hormone production by the ovaries. These medications include GnRH analogs, oral contraceptive pills, and progestins.
These drugs suppress estrogen production by the ovaries by inhibiting the secretion of regulatory hormones from the pituitary gland. As a result, menstrual periods stop, mimicking menopause. Nasal and injection forms of GnRH agonists are available.
Danazol is a synthetic drug that creates a high androgen and low estrogen hormonal environment by interfering with ovulation and ovarian production of estrogen. A newer approach to the treatment of endometriosis has involved the administration of drugs known as aromatase inhibitors and immunomodulators..
These drugs act by interrupting local estrogen formation within the endometriosis implants themselves. They also inhibit estrogen production in the ovary, brain, and other sources, such as adipose tissue.
The new selective progesterone receptor modulators may represent a valid hormonal treatment option. Therapeutic manipulation of the immune system through TNFalpha inhibitors may be beneficial in women with endometriosis. New pharmaceutical agents affecting inflammation, angiogenesis, and matrix metalloproteinase activity may prevent or inhibit the development of endometriosis.

Keywords: Medical Treatment, Endometriosis

I-47: Clomid Failure in Polycystic Ovarian Syndrome

Parsanejhad E

Shiraz University Medical Science, Shiraz, Iran
Email: parsame@sums.ac.ir

I-48: Cancer and Fertility Preservation

Rashidi B*, Iravani M

Vali-e-Asr Reproductive 1 Health Research Center Tehran University of Medical Sciences, Tehran, Iran

Recent advances in cancer therapy have given many patients hope and have lead to survival rates of approximately 70% treatments such as radiation, chemotherapy and surgery may have life altering implication.
For women and men of reproductive age, cancer therapies can often lead to ovarian damage and premature menopause both of which can have significant impact on current and future fertility.
Thanks to significant advances in reproductive medicine, there are technologies now available that can help to preserve fertility prior to undergoing cancer treatment.
The decision to preserve fertility in the face of cancer is difficult. Decisions regarding the timing of cancer treatment and disposition of the eggs or embryos, should the patient not regain the health needed to carry a pregnancy, are never easy and require the support of family and friends and the expertise of a wide range of health care specialists
Fertility preservation options in male patients:
Sperm cryopreservation. Sperm cryopreservation is effective, and oncologists should discuss sperm banking with appropriate patients, it is strongly recommended that sperm are collected prior to initiation of treatment because the quality of the sample and sperm DNA integrity may be compromised even after a single treatment session. Although planned chemotherapy may limit the number of ejaculates, intracytoplasmic sperm injection allows the successful freezing and future use of a very limited amount of sperm.
Hormonal gonadoprotection. Hormonal therapy in men is not successful in preserving fertility when highly sterilizing chemotherapy is administered.
Other consideration. Men should be advised of a potentially higher risk of genetic damage in sperm stored after initiation of therapy. Testicular tissue or spermatogonial cryopreservation and transplantation or testis xenografting have not yet been tested successfully in humans. Of note, such approaches are also the only methods of fertility preservation potentially available to prepubertal boys.
Fertility preservation options in female patients:
Embryo cryopreservation. Embryo cryopreservation is considered an established fertility preservation method because it has routinely been used for storing surplus embryos after in vitro fertilization.Approximately 2 weeks of ovarian stimulation with daily injections of follicle-stimulating hormone is required as must be started whitin the first 3 days of menstrual cycle.
Cryopreservation of unfertilized oocytes.
Cryopreservation of unfertilized oocytes is an option,

particularly for patients without a partner of those with religious or ethical objections to embryo freezing. Ovarian stimulation is required as described in the preceding section. Oocyte cryopreservation should be performed only in centers with the necessary expertise.
Ovarian tissue cryopreservation. ovarian tissue cryopreservation and transplantation procedures should be performed only in centers with the necessary expertise under scientific approved protocols that include follow-up for recurrent cancer. A concern with reimplanting ovarian tissue is the potential for reintroducing cancer cells, although in fewer than 20 procedures reported thus far, there are no reports of cancer recurrence.
Ovarian suppression. Currently, there is insufficient evidence regarding the safety and effectiveness of gonadotropin releasing hormone analogs and other means of ovarian suppression on fertility preservation. Women interested in this technique are encouraged to participate in clinical trials.
Ovarian transposition. Ovarian transposition (oophoropexy) can be offered when pelvic radiation is administered as cancer treatment because of the risk of remigration of the ovaries, this procedure should be performed as close to the radiation treatment as possible.
Conservative gynecologic surgery. It has been suggested that radical trachellectomy be restricted to stage IA2-IB disease with diameter less than 2 cm and invasion less than 10 mm. in the treatment of other gynecologic malignancies, interventions to spare fertility have generally centered on doing less-radical surgery and/or lower-dose chemotherapy with the intent of sparing the reproductive organs as much as possible.
Other considerations. Of special concern in breast and gynecologic malignancies is the possibility that fertility preservation interventions and/or subsequent pregnancy may increase the risk of cancer recurrence. Although several studies have not shown a decrement in survival or an increase in risk of breast cancer recurrence with pregnancy, the studies are all limited by significant biases, and concerns remain for some women and their physicians.
Fertility preservation methods are still applied relatively infrequently in the cancer population, limiting greater knowledge about success and effects of different potential interventions. Other than risk of tumor recurrence, less attention is paid to the potential negative effects (physical and psychological) of fertility preservation attempts.
Despite the facts that considered before, and other uncertainties in this
Despite the facts that considered before, and other uncertainties in this regard, cancer patients should be informed of options for fertility preservation and future reproduction prior to cancer treatment and this is not fully possible except by cooperation of cancer specialists, fertility specialist and the patients.

I-49: Molecular and Toxicology Evaluation of Patients with Endometriosis

Rozati R*, Giragalla SG, Maria T, Rahaban S

Mahavir Hospital & Research Center, Reproductive Medicine, Hyderabad, India
Email: drrozati@rediffmail.com

Objective: To estimate the role of dioxins and the etiology of patients with endometriosis. To determine the GSTM1 null (*0/*0) mutation and their possible impact in the pathogenesis of endometriosis.
Materials and Methods: Two hundred cases of endometriosis diagnosed by laparoscopy and one hundred and sixty six women without endometriosis were laparoscopically confirmed to be without endometriosis. Women age between 20 to 35 years were considered in this study from the year January 2005-March 2008. 200 (54.6%) as a study group and 86 (23.4%) sample control group 1 (C1), 80 (21.8%) as a control 2 (C2). Endometriosis was staged as minimal (rAFS stage I) in 90, mild (rAFS stage II) in 57, moderate (rAFS stage III) in 37, and severe (rAFS stage IV) in 16 patients. 6-10 ml heparinised Blood samples were collected from all the 200 cases and 166 controls (total=366) for DNA isolation and centrifuged (2500 r.p.m for 15 min) within 24 hours after collection. The serum (3-5 ml) was pooled and kept frozen at –20oC until the dioxins were analyzed. The extraction of dioxins using gas chromatography (GC) was divided into 5 phases. Extraction of dioxins was performed by the method described by Bruce et al with modifications by Rozati et al. The concentrated organic phases in phase four were pooled and dried under nitrogen gas. The sample was then re-suspended in hexane and then injected into the gas chromatograph. GC analysis carried out as per the instructions of the suppliers from Germany (Supelco Data , 1998), and in-house modifications done at Center for Cellular and Molecular Biology on GC-2010 series gas chromatograph (Shimadzu, Japan), equipped with a capillary column injection port. The concentrations of the dioxins (Di-n-Butyl, Butyl Benzyl, Di- n-Octyl, and Bis-2-ethyl Hexyl) were detected by the Gas chromatography (GC).
Results: Women with endometriosis showed significantly higher concentrations of dioxins compared with control group. We found that 26.8% of the cases with endometriosis and 14.7% of the controls had the GSTM1 null (*0/*0) genotype [odds ratio (OR = 2.12, 95% confidence interval (CI) = 1.045-4.314], which showed significant association (p=0.03) with endometriosis. The correlation between the concentrations of dioxins, GSTM1 null genotype and different severity of endometriosis was strong and statistically significant at $p<0.05$ for all four compounds and GSTM1 (DnBP: 0.44 SD (0.41); BBP: 0.66 SD (0.61; DnOP: 3.32 SD (2.17) and DEHP: 2.44 SD (2.17)) mgml-1.
Conclusion: The study results suggest that women having higher concentration of dioxins and GSTM1 null (*0/*0) polymorphism might have an increased susceptibility of endometriosis.

Keywords: GSTM1, Endometriosis, Dixins, Gas Chro-

matography, Polymorphism

I-50: Isolation and Phenotypic Characterization of Human Endometrial Epithelial and Stromal Cells

Rozati R*,Giragalla SG, Maria T, Rahaban S

Mahavir Hospital & Research Center, Reproductive Medicine, Hyderabad, India
Email: drrozati@rediffmail.com

Objective: To isolate the human endometrial epithelial and stromal cells. To determine the cellular phenotypes of the isolated endometrial epithelial and stromal cells
Materials and Methods: 200 infertile couples were screened for infertility between June 2006- April 2008. Eighty five women of south Indian origin with minimal, mild, moderate, and severe (Stages I-IV) endometriosis were diagnosed by laparoscopy. All women under went trans vaginal ultra sound scan, followed by laparoscopy to confirm the diagnosis (revised AFS I -35; revised AFS II- 26; revised AFS III-14; and revised AFS IV- 10). One hundred thirty five controls were included in this study who is undergone hysterectomy with proven fertility. Endometrial tissue has collected from ovulating women in HEPES- buffered Dulbecco modified Eagle medium-Hams F- 12 (DMEM/F-12: Invitrogen, Carlsbad, CA) with 1% antibioticantimycotic solution. And 5% new born Calf serum (NCS; CSL Ltd Parkville, VIC, Australia), stored at -48o C, and processed within 2-18hrs. A single-cell suspension of endometrial cells was obtained using enzymatic digestion and mechanical means adapted from Gargett et al. Beaded endometrial epithelial cells and purified endometrial stromal cells were seeded in triplicate at clonal density, 500 and 300 cells/cm2, respectively, into 60-mm Petri dishes (Becton Dickinson Labware, Bedford, MA) coated with gelatin (Sigma-Aldrich, St. Louis, MO) for epithelial cells or fibronectin (10 mg/ml; Becton Dickinson Biosciences) for stromal cells and cultured in serum medium (SM), containing bicarbonatebuffered DMEM/F-12 medium, 10% FCS (CSL Ltd.), 2 mM glutamine (Invitrogen), and antibiotic-antimycotic. Endothelial cell growth factor, 20 mg/ml (Roche Diagnostics) was also included for epithelial cell culture in SM.
Results: We have successfully established epithelial and stromal cell monolayer cultures from both normal endometrium and endometrioma biopsies. A typical yield of stromal cells from dissected endometrioma specimens is 2-4 X106 cells/g wet wt of cyst wall, approximately half the yield from 1 g proliferative endometrium. Epithelial cells are more difficult to quantify because they are isolated as glands and not as dispersed cells. However, the yield of endometrioma glands was invariably less than that from endometrial biopsies. Normal endometrial biopsies obtained in the proliferative phase yielded healthy epithelial and stromal cultures in 6 of 7 attempts. Under phase contrast microscopy, the epithelial cells were characteristically intercalated and tadpole-shaped, with similar morphology observed for the normal endometrial and endometriosis cell culture.
Conclusion: The present study is the first to demonstrate that human endometrium contains a small population of epithelial and stromal cells. And demonstrate that highly purified epithelial and stromal cells cultured from normal endometrial and endometriosis tissues express the same phenotypic and functional markers as their in-vivo counterparts. These cultures provide useful models to identify endometriosis-specific cell products that contribute to the pathogenesis of this disorder.

Keywords: Endometrial Stromal Cells, Epithelial Cells, Endometrioma, Endometriosis

I-51: Office Hysteroscopy in Infertility Diagnosis and Treatment

Sadri S.

Rasht Family IVF Center, Rasht, Iran
Email: saeideh.sadri42@gmail.com

For the recent years, gynecologists have been usining the hysteroscope in the office to diagnose a variety of conditions that can be responsible for symptoms such as abnormal uterine bleeding, recurrent miscarriage, infertility, and post menopausal bleeding. The most common lesions found during diagnostic office hysteroscopy include cervical and uterine polyps, submucous myoma, uterine septums, intrauterine adhesions, endometrial hyperplasia and endometrial cancer.
W hen benign intrauterine and intracervical lesions are diagnosed by office hysteroscopy, patients are scheduled to be treated within the operating room. There are many reasons for this including a greater availability of anesthesia, and the risks and discomfort for patients within the office setting. In addition, physicians are concerned about the cost of equipment necessary for performing office hysteroscopy.
It is very important to understand the cost savings of performing procedures in the office setting as opposed to the hospital operating room. For most of the procedures in infertility treatment it is possible to decrease this cost.
In office hysterosocpic procedures that can be performed with a mini hysteroscope, the majority of these procedures can be performed with either no anesthesia or oral NSAIDs.
We will discuss about the vaginoscopic option of hysteroscopy and feasibility of performing this procedure in office without anesthesia, for infertility diagnosis and treatment.

I-52: 3D-4D Ultrasound in Uterine Anomalies

Salamati M

Reproductive Imaging Division, Department of Endocrinology and Female Infertility; Royan Institute, Tehran, Iran

I-53: Treatment Options in Polycystic Ovarian Syndrome

Salehpour S

Obstetrics and Gynecology Department, Shahidbeheshti University of Medical Sciences, Tehran, Iran
Email: saghar_salehpour@yahoo.com

Treatment of pcos has different lines:
1-Infertility
2-Metabolic syndrome
3-Cosmetic problems
4-Obesity
5-Endometrial hyperplasia

First line of treatment includes diet, weight loss, and exercise and second line is pharmacologic treatment:
1-Improvement of peripheral insulin resistance
2-Decrease glucose absorption
3-Decrease hepatic glucose production
4-Anti androgens
5-Ovulation induction

I-54: The Perpetual Question of the Uterine Myomas; to Treat or not to Treat?

Sawalhe S

I-55: Development of Male Contraceptive Methods in India

Sharma RS

Indian Council of Medical Research, Ansari Nagar, New Delhi-110 029
Email: radheyss@gmail.com

The steadily expanding population in our country is of great concern due to its impact on health and societal dynamics. Contraceptive prevalence rate in our country is 56.3% where as contribution of male to this figure is only 6.2%. One of the main reasons for low contraceptive prevalence rate of male is because of limited choice of contraceptives for male. There are only two methods available for male in the cafeteria of contraception and i.e. condom and vasectomy. Therefore, in order to expand the contraceptive options for male scientists have been experimenting to develop new contraceptive methods. To develop a male contraceptive the current research is directed mainly towards the following: (i) development of anti spermatogenic agents to suppress sperm production; (ii) prevention of sperm maturation; (iii) prevention of sperm transport through vas deferens or rendering these sperm infertile and (iv) prevention of sperm deposition.
Scientists have been experimenting with various steroidal/non-steroidal regimens to suppress testicular sperm production. One of the main limiting factors in developing male contraceptive regimen is non-availability of a long acting androgen as all anti-spermatogenic agents tested or under testing decreases circulating testosterone levels. Long acting progestogen and long acting androgen would have advantage over an androgen-alone modality as the dose of an androgen required would be much smaller in the combination regimen, thereby decreasing the adverse effects of high steroid load. A number of combination regimens using progestogen or GnRH analogues combined with androgen are under going trials. Research work in the field of prevention of sperm transport through vas deferens or rendering these sperm infertile has made significant advances in our country. A copolymer of styrene and maleic anhydride now called as RISUG® (Reversible Inhibition of Sperm under Guidance) has undergone extensive testing in animals and at pre clinical level. Efficacy and safety of RISUG® has also been established through Phase-I, Phase-II, limited phase III clinical trials. Phase-III clinical trial is currently going on at four centers in the country which will be extended to six more centers shortly. It is hoped that RISUG would be available in the near future as an indigenously developed injectable intra-vasal male contraceptive. Efforts are also being made to popularize non-scalpel vasectomy (NSV). To increase the acceptability and effectiveness of condom efforts have been made to collect correct specifications required for Indian man. Efforts are also on going to develop new generation condom suites to our population.

I-56: The outcome of In Vitro Fertilization/Intra Cytoplasmic Sperm Injection in EndomeTriosis-Associated and Tubal Factor Infertility

Shahrokh Tehrani Nejad E[1,2,3*], Rashidi B[2,3], Larti A[2], Ezabadi Z[1], Jahangiri N[1], Azimi Nekoo E[2]

1. Endocrinology and Female Infertility Department, Reproductive Medicine Research Centre, Royan Institute, ACECR, Tehran-Iran
2. Obstetrics and Gynecology Department, Tehran University of Medical Sciences, Tehran-Iran
3. Vali-e-asr Reproductive Health Research Centre, Tehran University of Medical Sciences, Tehran-Iran
Email: tehraninejad@royaninstitute.org

Background: Endometriosis is one of the most challenging disease that constitute 20%-40% of women searching for their infertility diagnosis.
Objective: This study was undertaken in order to compare the outcome of IVF/ICSI (In Vitro Fertilization/ Intra Cytoplasmic Sperm Injection) in women with endometriosis, using tubal factor infertility as controls.
Materials and Methods: From 2005 to 2006 a retrospective study was carried out in patients with endometriosis (n=80) and tubal infertility (n=57) after treatment with IVF/ICSI. The main outcome measures were ovarian responsiveness, implantation, pregnancy, live birth and twin birth rates. Appropriate statistical analysis was performed using $\chi 2$ and student t-tests.
Results: The mean duration of infertility was significant-

ly lower in women with moderate to severe endometriosis than the tubal factor group ($p<0.01$). There was also a significant difference in history of ectopic pregnancy between endometriosis and tubal factor groups ($p<0.001$). However no differences were found in mean number of ampuls of hMG, duration of HMG injection, number of MII oocytes, number of embryo transferred, implantation and pregnancy rates, live birth and twin birth rates between compared groups.
Conclusion: Our results suggest that ovarian response and uterine receptivity are not impaired in women with endometriosis versus tubal infertility.

Keywords: Endometriosis, IVF/ICSI Outcome, Tubal Factor, Infertility

I-57: Endometriosis and Infertility

Ussia A[1*], Koninckx Ph.R[2]

1. Villa Giose, Crotone, Italy
2. University Hospital Gasthuisberg, 3000 Leuven, Belgium
Email: anastasia.ussia@gmail.com

Endometriosis can present as subtle lesions, typical lesions, cystic ovarian endometriosis and deep endometriosis.
To the best of our knowledge, subtle endometriosis has never been shown to be related neither to infertility not to pain. We do not consider subtle lesions as pathology but rather as a physiologic event occurring intermittently in all women. Hence theoretically treatment can be considered unnecessary; practically however, we prefer to vaporize with a CO2 laser since easy and without risks.
Typical lesions are associated with infertility and the monthly fecundity rate is reduced below 10%. The pathophysiology of the associated infertility is unknown and ranges from abnormal uterine contractions (the Archimetra concept), to reduced endometrial receptivity and to absence of real ovulation ie the luteinized unruptured follicle concept. The latter is clearly associated with typical lesions, and in primates repetitive. Treatment of typical lesions should be done by excision, either by Co2 laser of with cold scissors. Postoperative cumulative pregnancy rates typically are some 70% within 1 year with a monthly fecundity rate around 10%. Whether treatment is effective in enhancing the pregnancy rates is still debated since the 2 available randomised controlled trials (Gruppo Italiano and the Endocan study) are contradictory. The Endocan study moreover can be criticized for its design.
Cystic ovarian endometriosis, in addition is associated with adhesions and mechanical infertility. Only Surgery should consist of cyst wall excision which is associated with a lower recurrence rate than focal treatment. Care should be taken not to destroy the ovary by removing part of it or by coagulating the hilus. Results are similar to those obtained for typical lesions
For deep endometriosis, the association with infertility is less clear. These women however require surgical excision because of severe pain. Results after treatment also are similar to typical endometriosis
Medical treatment of endometriosis is ineffective in improving infertility.

I-58: Laparoscopic Surgical Treatment for Infertility

Ussia A[1*], Koninckx Ph.R[2]

1. Villa Giose, Crotone, Italy
2. University Hospital Gasthuisberg, 3000 Leuven, Belgium
Email: anastasia.ussia@gmail.com

Before detailing the possibilities of laparoscopic surgical treatment a clear understanding of the energy modalities is required, ie electrosurgery, CO2 laser and harmonic scalpel. Secondly, especially in infertility, the 'see and treat' principle is important, ie during the diagnostic laparoscopy almost all surgical treatment should be feasible. Finally the relative advantages and disadvantages, of performing systematically a diagnostic laparoscopy early during infertility investigation, should be considered. We will not discuss the pro and con's of performing systematically a laparoscopy or instead a THL in those women without suspicion of pathology.
The preoperative work-up aims at identifying those women who need a bowel preparation and those women who need a ureter stent. When a deep nodule is suspected, clinically or by ultrasound, therefore a contrast enema and IVP are mandatory and a bowel preparation should be given. We also favor to give a bowel preparation for women who have been operated several times beforehand.
During laparoscopic surgery any pathology will be treated. This varies from the treatment of the various forms endometriosis, to adhaesiolysis, myomectomy, salpingostomy and ovarian drilling.
Endometriosis has already been discussed.
Adhaesiolysis and adhaesion reformation is a controversial topic, for which none of the available antiadhaesive treatments has been validated. Yet today we expect that soon effective treatments will become available through additional pneumoperitoneum conditions ie adding small amounts of oxygen and cooling.
The relationship between myoma's and infertility is unclear. It is widely accepted however that submucoal myoma's should be removed by hysteroscopy, and that the larger subserous or pedunculated myoma's can cause mechanical infertility.
Salpingostomy is the treatment of choice for hydrosalpinges with cumulative pregnancy rates around 60%. In women with thickwalled hydrosalpinges or with absence of mucosal folds at salpingoscopy of in case of tuberculosis, a salpingectomy should be performed.
Ovarian drilling restores ovulation in some 80% of PCO women. Only 50% get pregnant and the drilling associated adhesions and infertility remains a topic of debate. Recently, drilling by THL has been introduced and initial

data suggest that after 'underwater' drilling adhaesions are virtually absent .
Finally surgery should be balanced with the IVF. Today however, surgery still is the method of choice provided performed adequately

Keywords: Endometriosis Can Present as Subtle Lesions, Typical lesions, Cystic Ovarian Endometriosis and Deep Endometriosis

I-59: Female Genital Tract Tuberculosis: Hysterosalpingographic Appearances

Vosough A, Ahmadi F, Zafarani F

Reproductive Imaging Division, Reproductive Medicine Research Center, Department of Endocrinology and Female Infertility, Royan Institute, ACECR, Tehran, Iran

Genital tract tuberculosis is an important cause of infertility in developing countries where hysterosalpingography (HSG) remains the initial diagnostic procedure in the assessment of tubal and peritoneal factors leading infertility.
The primary focus of genital tuberculosis is the fallopian tubes, and affecting of endometrium is secondary to down passage of bacteria into the uterine cavity.
Tuberculosis gives rise to varied appearances on hysterosalpingography (HSG). These features vary from non-specific changes such as hydrosalpinx, evidence of endometritis, intrauterine adhesions, septations, an asymmetric uterine cavity, and other evidence of reduction in the uterine luminal volume to specific appearances such as "beaded tube", "golf club tube" , "pipestem tube", "cobblestone tube", "leopard skin tube", "collar-stud abscess", " the tuberculosis T-shaped" uterus, and the "pseudounicornuate" uterus.
All of these features can be depicted on hysterosalpingography (HSG), which is the gold standard in the investigation of female genital tract tuberculosis.

Genetics

I-60: Genetic Aspects of Recurrent Miscarriage

Almadani N

1. Clinical Genetic Department, Karimnejad- Najmabadi Pathology & Genetic Center, Tehran, Iran
2. Genetic Department, Reproductive Medicine Research Center, Royan Institute, ACECR, Tehran, Iran
Email: navidalmadani@yahoo.com

Recurrent Pregnancy loss is the miscarriage of two or three Consecutive Pregnancies in The first or early second trimester. Although approximately 10-15% of all recognized Pregnancies result in miscarriage, less than 5% of women will experience two consecutive miscarriage , and only 1% experience Three or more.In recurrent Pregnancy loss, Current Practice often Fails To make a diagnosis , as the fetal causes of Pregnancy loss are usually ignored and only the maternal factors are assessed. The maternal Causes are well known and include: Uterine factors, Infections, Autoimmune syndromes, endocrine abnormality, alloimmune factors and possibly hereditary thrombophilia. However, all assessments of these factors have been confounded by the presence of abnormal embryos that may themselves by incompatible with life. The fetal Causes of embryo loss include structural malformations that are incompatible with life, and Chromosomal aberrations. Unfortunately, no explanation is found in 50% to 70% of Couples with recurrent pregnancy loss. About 5% of Couple with recurrent Pregnancy losses have A Chromosomal abnormality and translocation is the most common inherited chromosomal abnormality. Although a parent who carries a translocation is frequently normal, their embryo may receive too much or too little genetic material. Couples with translocation or other specific chromosome defects may benefit from Pre-implantation genetic diagnosis. But in fact 60% or more of early miscarriages may be caused by a random chromosomal abnormality, usually a missing or duplicated chromosome. However, 89% of human recurrent miscarriages occur in the first trimester that this stage is too early to be diagnosed as normal or abnormal body structure on ultrasound. These defects included neural tube defects, Poly–syndactyly, cleft lip and cleft hand. These malformations are usually associated with a normal karyotype.
Single gene disorders associated with recurrent miscarriage are myotonic dystrophy, factor V leiden mutation. The factor V leiden mutation is the most common genetic predisposition to thrombosis but its carrier frequency in the white population is 3-4 %.

I-61: Prenatal Diagnosis Techniques to Determine the Health and Condition of an Unborn Fetus

Houshmand M

National Institute for Genetic Engineering and Biotechnology, Special Medical Center, Tehran, Iran
Email: Massoudh@nigeb.ac.ir

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both. Congenital anomalies account for 20 to 25% of perinatal deaths. Specifically, prenatal diagnosis is helpful for:
• Managing the remaining weeks of the pregnancy
• Determining the outcome of the pregnancy
• Planning for possible complications with the birth process
• Planning for problems that may occur in the newborn infant
• Deciding whether to continue the pregnancy

• Finding conditions that may affect future pregnancies
There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each of them can be applied only during specific time periods during the pregnancy for greatest utility. The techniques employed for prenatal diagnosis include:
• Ultrasonography
• Amniocentesis (for biochemical and genetic analyzing)
• Chorionic villus sampling (for biochemical and genetic analyzing)
• Fetal blood cells in maternal blood
• Maternal serum alpha-fetoprotein
• Maternal serum beta-HCG
• Maternal serum estriol
Here we discuss about the most molecular genetic tests to detect abnormalities in fetus.

I-62: The Genetic Role of Male Side on Unexplained Repeated Pregnancy Loss

Kalantar SM[1*], Sheikhha MH[1], Seyed Hassani SM[1,3], Fazli H[1], Rasti N[1], Khodai H[1], Aflatonian A[1], Dehghani R[1], Pakravesh[2]

1. Research & Clinical Centre for Infertility, Yazd Medical Sciences University, Yazd, Iran
2. Aban Hospital, Tehran, Iran
3. NCGE Tehran –Iran
Email: smkalantar@yahoo.com

Objective: The role of male in repeated pregnancy loss (RPL) is not described very well. To evaluate the role of Y chromosome microdeletion in the case of couples had problem with RPL in-group of unknown cause compared with couples with male factor infertility.
Materials and Methods: A controlled clinical study was designed in our centre. In total, 100 men from male factor infertility and 25 fertile men with at least one child 25 men with RPL were recruited in the study.DNA was extracted from Peripheral blood sample. In each sample, six sequence-tagged-sites (STS) according to the europian protocol and four other STSs in the proximal AZFc region namely; DYS262, DYS220, DYF8551, and DYF8651 were studied by polymerase chain reaction (PCR).
Results: Eight men tested evaluated had Y microdelation in at least one of the six segments from Europian protocol (8%) VS non in RPL group. Five men with history of at least 3 PL in their wife tested for 4 STS in AZFc region had microdelation (20%) but non of those other STS. In the inferile group had not any microdeletion related to the 4 STS from AZFc region. In the third group only one microdeletion was found.
Conclusion: From our results it could concluded that in case of couples with un explained PRL it may help to test these four STSs on Y chromosome to recognize the cause of PL.

Keywords: Repeated Pregnancy Loss, Y Chromosome, Microdeletion, AZFC Region

I-63: Contamination During PGD: How We Can Deal with?

Modarresi MH

I-64: Single Gene Point Mutation Analysis Using OligoArray and HairLoup Technique

Moncef B

ATL R&D Laboratory, Paris & UNILABS Laboratories, Geneva
Email: Atl78@aol.com

I-65: Genome Profiling of Ovarian Adenocarcinomas Using Pangenomic BACs Microarrays

Moncef B

ATL R&D Laboratory, Paris & UNILABS Laboratories, Geneva
Email: Atl78@aol.com

A number of strategies have been used for early detection of ovarian cancer and for follow-up. Tumor marker CA 125 and trans vaginal ultrasound are the most common such procedures. Gene expression and proteomics arrays were recently applied to identify markers that can be used to detect ovarian cancer at early stage . Ovarian cancers in stage I seem to exhibit different genotype and phenotype than metastasis stage III-IV. For example, a study by Simon et al. showed that breakpoints in regions 1p3 and 11p1 are important early events and distinguish a class of tumors associated with poor prognosis in ovarian adenocarcinoma. Thus, we can improve clinical management of ovarian cancer patients by better understanding genomic changes.
Some authors have utilized fluorescence in situ hybridization, classical comparative genomic hybridization and Multiplex ligation-dependent probe amplification to screen genome abnormalities in ovarian cancer tissues. For ovarian cancer profiling studies using micro arrays, high resolution single nucleotide polymorphism array was applied for micro deletions and amplifications analysis in serous carcinomas and to elucidate the pathogenesis of ovarian carcinoma (Nakayama et al 2007). Expression and proteome micro arrays were also applied to investigate the down-up regulation and the expression of the most involved genes in the molecular pathways of ovarian cancer. For molecular karyotyping Array CGH (A-CGH) methods are superior to FISH in not requiring suitable nuclear preparations and in not being limited to probes used. They are also superior to routine metaphase CGH because of their higher resolution, easier interpretation and hold the promise and routine diagnostic tool to identify visible and submicroscopic chromosome abnormalities. we applied A-CGH technique for genome profiling patients with ovarian cancer after tissues biopsy or aspiration (caserta et al 2008) and compared our data to the results reported by the literature using different cytogenetics techniques.

Stem Cells

I-66: Cell Therapy Clinical Trials at Royan Institute

Aghdami N[1*], Baharvand H[1], Namiri M[1], Malekzadeh R[2], Ahmadi H[3], Pournasr B[1], Zafarghandi MR[4], Taghiabadi E[1], Javadi MA[5], Ebrahimi M[1], Khodadai L[1], Shafian S[1], Malek Hosseini SA[6], Ravari H[4], Nikeghbali S[6], Mohamadnejad M[2], Baradaran Rafiei A[5]

1. Department of Stem Cells, Cell Sciences Research Center, Royan Institute, ACECR, Tehran, Iran
2. Digestive Disease Research Center, Medical Sciences/ Tehran University, Tehran, Iran
3. Tehran University of medical sciences, Tehran heart center, Tehran, Iran
4. Tehran University of medical sciences, Vascular Surgery department, Sina Hospital, Tehran, Iran
5. Department of Ophthalmology, Labbafinejad Medical Center, Shahid Beheshti Medical University, Tehran, Iran
6. Organ transplantation center, Namazee Hospital, Shiraz University of Medical sciences, Shiraz , Iran
Email: nasser.aghdami@royaninstitute.org

Adult stem and progenitor cells from various sources have been shown to augment the functional effect for treating cardiac, liver, lung and neurological diseases. Clinical trials have confirmed that autologous cell therapy using bone marrow derived or circulating blood derived progenitor cells is safe and provides beneficial effects. The Royan institute seeks discoveries and methods that will accelerate cell-based therapies and brings about a whole new field of medicine. Here I review the Institute experience in patients with acute myocardial Infarction, Liver cirrhosis, vitiligo, limbal stem cell deficiency and peripheral artery diseases. There investigations showed, Royan institute has capability of moving basic pre-clinical discoveries in stem cell field, into clinical phase of development for initial evaluation in patients.

I-67: Embryonic Stem Cell Research at Royan Institute

Baharvand H[1,2,*], Hosseini Salekdeh S.Gh[1], Zare Mehrjerdi N[1], Taei A[1], Nasr-Esfahani MH[3], Totonchi M[1], Hatami M[1], Kiani S[1], Pakzad M[1], Karbalaie Kh[3], Mollamohammadi S[1], Farrokhi A[1], Shahsavani M[1], Rezaei M[1], Seifi A[1], Azizi H[1], Hemessi K[1], Pirouz M[1], Tanhaei S[3], Pournasr B[1], Moradmand B[1], Pouya A[1], Nemati Sh[1], Karamali F[1], Rabbani R[1]

1. Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
3. Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Isfahan Campus, Isfahan, Iran
Email: Baharvand50@yahoo.com

Embryonic stem cells derived from a preimplantation embryo appear to have an unlimited capacity to self-renew in cell culture, and they are also able to differentiate into hundreds of adult cell types. Human embryonic stem cell lines offer a platform technology that has the potential to elucidate the molecular mechanisms that determine adult cell fate, generate cellular models for discovery of new drugs, and create populations of differentiated cells for novel transplantation therapies. Here, our studies about embryonic stem cell establishment, differentiation into cell types representing the three embryonic germ lineages, and their potential applications will be summarized.

I-68: From Neural Stem Cells to Brain Repair

Brüstle O

Institute of Reconstructive Neurobiology, University of Bonn Medical Center, Bonn, Germany
Email: oliver.bruestle@uni-bonn.de

I-69: From Pluripotent Cells to Stable Neural Stem Cells

Brüstle O

Institute of Reconstructive Neurobiology, University of Bonn Medical Center, Bonn, Germany
Email: oliver.bruestle@uni-bonn.de

I-70: Modeling Disease with Human Pluripotent Cells

Cowan Ch

Cardiovascular Research Center, Boston, USA
Email: CCOWAN1@PARTNERS.ORG

I-71: Understanding the Differentiation Propensity of Human Embryonic Stem Cells

Cowan Ch

Cardiovascular Research Center, Boston, USA
Email: CCOWAN1@PARTNERS.ORG

I-72: Genetic Modification of Human Embryonic Stem Cells

Cui W

Institute of Reproductive and Developmental Biology, Imperial College London, UK.
Email: wei.cui@imperial.ac.uk

The isolation and culture of human embryonic stem cells (hESCs) has opened up new opportunities not only for regenerative medicine and other biomedical applications but also for the study of early human development, signalling pathway networks and mechanisms of human diseases. However, it is remain unclear about the factors

important for the maintenance of undifferentiated hESCs in vitro and factors /signalling pathways leading hESCs differentiation to specific lineages. Genetic modification of hESCs will facilitate our investigation to achieve these goals. For example, incorporation of fluorescent reporter genes in a lineage specific expression pattern will enable more effective screening to identify molecules regulating specific cell fate commitment. Ectopic expression, or silencing, of key developmental genes/ will enhance our understanding their function in hESCs self-renewal or differentiation. We have generated hESCs reporter cell lines expressing green fluorescent protein (GFP) under the control of ES cell (e.g. Oct4) or lineage specific (e.g. α-fetoprotein, AFP for endodermal lineage) promoters using either chemical transfection or lentiviral transduction techniques. Using these reporter cell lines, we have compared effect of bFGF in regulation of human ES cell self-renewal, studied Oct4 expression in regulation of hESCs differentiation and development of better system to differentiate hESCs to hepatic cell fate.

I-73: Efficient Differentiation of Human Embryonic Stem Cell to Functional Hepatocytes

Cui W

Institute of Reproductive and Developmental Biology, Imperial College London, UK
Email: wei.cui@imperial.ac.uk

Human embryonic stem cells (hESCs) are able to replicate indefinitely and to differentiate into most, if not all, cell types of the human body in vitro and in vivo. These unique properties make them an unlimited cell resource for a variety of biological, biomedical and medical applications. Most of applications of hESCs require efficient and regulated differentiation of hESCs to defined cell types. Hepatocytes, the primary cells of the liver, have attracted particular attention as the liver plays a central role in multiple functions of the human body. One of our research interests is to establish a differentiation system by which hESCs can be efficiently differentiated into hepatocytes. We particularly interested in the generation of hepatocytes which could be valuable for assessing the toxicity of new drugs, as liver is the primary tissue involved in the metabolism of drug compounds and is among the most common tissues affected by drug toxicities. The most commonly used method for differentiating ES cells is by initiating the differentiation via embryoid body (EB) formation, which resembles to a certain degree the early embryo development in vivo. However, individual cells in the EBs receive variable autocrine and paracrine signals according to their location in the EBs as well as the size of the EBs, which subsequently can lead to different cell fates. As a result, the differentiated cells by this approach are often heterogenous, possibly containing all three germ layers. In addition, it is difficult to dissect signalling pathways required for certain cell type differentiation with this method. Therefore, recent progresses have been made to differentiate ES cells in adherent culture with media supplemented with a cocktail of growth factors and cytokines which promote differentiation of hESCs to a more enriched populations of desired cell types. We have applied this strategy to hepatocyte differentiation and efficiently differentiate hESCs to definitive endoderm, then to the hepatocytes. The differentiation process of hESCs recapitulates the liver development in vivo and hESC-derived hepatocytes are able to carry out a range of hepatocyte functions, particularly expressing several members of cytochrome P450 isozymes which are capable of converting the substrates to metabolites.

I-74: Aging of Hematopoietic Stem Cells: Mechanisms and Molecules

De Haan G*, Gerrits A, Bystrykh LV, Kamminga LM, Weersing E, Dontje B

Department of Cell Biology, University Medical Center Groningen, Groningen, the Netherlands.
Email: g.de.haan@med.umcg.nl

Hematopoietic stem cell (HSC) self-renewal is driven by both intrinsic and extrinsic factors, but the molecular mechanism specifying whether developmental potential is lost or retained during asymmetric cell divisions is unknown. Serial transplantation studies have clearly indicated that self-renewal potential of HSCs is limited, but the molecular mechanism responsible for a decline of stem cell functioning after replicative stress remains unknown. No genes have been found that counteract stem cell senescence. We used hematopoietic stem cells (HSCs) to screen genes involved in the process of cellular aging, and identified Enhancer of zeste homolog 2 (Ezh2), a Polycomb group protein (PcG) involved in histone methylation and deacetylation. Different members of the PcG gene family have recently been implicated to play a role in hematopoietic stem cell self-renewal but the potential function of Ezh2 in long-term repopulating HSCs has not been investigated. Ezh2 was expressed in freshly isolated HSCs, but downregulated during differentiation-inducing cultures. Whereas normal HSCs were rapidly exhausted after serial transplantations, retroviral overexpression of Ezh2 completely conserved long-term repopulating potential. Animals that were reconstituted with three times serially transplanted control bone marrow cells all succumbed due to hematopoietic failure. In contrast, similarly transplanted Ezh2-overexpressing stem cells restored stem cell quality to normal levels and showed normal blood cell values. Strikingly, a graft of only 50,000 5-times serially passaged Ezh2 cells provided chimerism levels >80% in competitive transplant experiments. These 5-times serially transplanted cells also engrafted in unirradiated normal recipients. In addition, with each serial transplant the white blood cell count and bone marrow and spleen cellularity of recipients of Ezh-transduced cells gradually increased, resulting in a very delayed (>1,5 years) myeloproliferative disease. In the

bone marrow of these animals an increased % of myeloid blasts was observed. In the spleen extensive extramedullary hematopoiesis was observed, with massive erythroid infiltration. In some animals high numbers of circulating nucleated erythroblasts were observed in the peripheral blood. Intriguingly, GFP expression driven from the retroviral transgene also increased with time, suggesting generalized loss of methylation or selection for clones that expressed highest Ezh2 levels. We previously identified a quantitative trait locus (QTL) on chromosome 18 that was associated with variation in stem cell pool size between C57BL/6 and DBA/2 strains of mice. Strikingly, variation in Ezh2 levels was largely accounted for by the exact chromosome 18 locus. In a 'genetical genomics' screen we identified novel putative Ezh2 target or partner stem cell genes that were all associated with chromatin modification. We confirmed the altered expression level of a large number of these transcripts in purified Ezh-transduced stem cells isolated from transplanted recipients. Our data suggest that stabilization of the chromatin structure preserves HSC potential after replicative stress. However, repression of stem cell senescence results in pre-leukemic myeloproliferation. Ezh2 is a key factor in balancing senescence and selfrenewal pathways in stem cells.

I-75: The Genome-Wide Identification of Master Regulators of Transcriptional Networks During Hematopoietic Development

Gerrits A, Bystrykh L, Weersing E, Dontje B, Li Y, Tesson B, Breitling R, Jansen R, De Haan G*

1. Department of Cell Biology, University Medical Center Groningen, Groningen, the Netherlands
2. Department of Bioinformatics, University of Groningen, the Netherlands.
Email: g.de.haan@med.umcg.nl

A fundamental problem in biology is how a single genome can lead to widely different cellular phenotypes. An illustrative and clinically relevant example is the generation of all mature blood cells types from a small population of hematopoietic stem cells. Identification of gene networks specifying stemness is of major relevance for the emerging fields of tissue engineering and regenerative medicine. We developed a genetical genomics approach as a tool to dissect networks of interacting genes that specify cellular function in four developmentally distinct hematopoietic cell stages. We evaluated genome-wide RNA transcript expression in highly purified Lin-Sca-1+c-kit+ multilineage cells, committed Lin-Sca-1-c-kit+ progenitor cells, erythroid Ter119+ and myeloid Gr1+ precursor cells isolated from a large pedigree of genetically related and fully genotyped BXD recombinant mouse strains. Variation in transcript abundance across all strains and in all cell types was assessed by Illumina Sentrix Mouse-6 chip technology, interrogating ~47,000 probesets mapping across of the genome. For each variably expressed transcript genetic linkage analysis identified a quantitative trait locus that affects variation in expression levels of the corresponding gene (eQTL). These eQTLs map in the vicinity of their target gene (cis-regulation), or map elsewhere in the genome (trans-regulation).
Complex transcript profiles for each cell type have been dissected into more simple individual gene networks, each consisting of transcripts whose variation in expression levels are regulated in trans by a single genomic locus. We identify highly cell stage-specific genomic loci that regulate variation in abundance of hundreds of transcripts. Strikingly, many genes that are equally expressed in distinct cell populations are nevertheless often controlled by very separate genetic loci. We generated gene regulatory maps for all four cell types and show that these regulatory maps are more discriminative than gene expressions patterns.
We predict that members of each network are functionally related, for example involved in a common biochemical pathway. We focus on those networks which exclusively operate in stem cells, identified by 'subtractive eQTL analysis'. Comparing those networks in primitive stem cells, multilineage progenitors, and in committed myeloid and erythroid cells, allowed identification of QTLs responsible for the corresponding lineage commitment.

I-76: 17 years experience of Hematopoietic Stem Cell Transplantation in Iran

Ghavamzadeh A, AliMogaddam K*, Khatami F, Jalili M, Masnavi A, Kashanpour A, Alimohammadi A, Eini E, Jahani M, Iravani M, Mousavi SA, Bahar B, Bibordi E, Khodabande A

Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Dr. Shariati Hospital, Tehran, Iran.
Email: alimgh@ams.ac.ir

Hematology-Oncology and Stem Cell Transplantation (SCT) Research Center related to Tehran University of Medical Sciences located in Shariati Hospital. Our center activities have started in 1991 in order to help needful patients and augment new data to reach new aspects of therapeutic trials. Also it is one of the greatest bone marrow transplantation centers in word and is the second center in the word based on the transplanted thalassemic patients. Also this center is doing scientific activities, so that it has presented over 250 assays in international congresses and also more than 150 Thesis has been performed under our professor's observations. Since the year 1991-when bone marrow transplantation was performed for the first time on three patients with Acute Myelogenous Leukemia(AML), Acute Lymphoblastic Leukemia (ALL) and Ewing Sarcoma- 2376 Hematopoietic Stem Cell Transplantation (HSCT) have been performed in patient with different diseases .There are 1538 cases that have received allogeneic HSCT and 703 cases that have received autologous HSCT. The first peripheral blood

Hematopoietic stem cell transplantation was performed in 1997 and since then, there are 1840 patients were done with this method. The first cord blood Hematopoietic Stem Cell Transplantation was performed in 1998 and since then there are 14 patients received HSCT from cord blood. We had 121 patients with Cellular Therapy (a technology that relies on replacing diseased or dysfunctional cells with healthy, functioning ones) for post MI, Thalassemia major, Multiple Sclerosis, Cirrhosis, Head of Femour Necrosis and Renal Cell Carcinoma.

Recently, new methods have been used like low intensity conditioning regimen (non myeloablative) and Donor Lymphocyte Infusion (DLI).This center is one of the International Blood and Marrow Transplantation Registry (IBMTR) and European group of Blood and Marrow Transplantation (EBMTR) member and in accompanies with these associations, is gathering the patient's databases who have been undergone transplantation; and cooperate with these centers in scientific and research fields. So that gives help to researchers for a better understanding of transplantation and invent new therapeutic methods. Our center is the member of Asian Pacific Cancer Center (APCC) and also we are collaborating with Blood and Cancer Associations such as American Society of Hematology (ASH), International Society of Hematology (ISH), and European School of Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO), and other centers. The plans and aims include protraction of cytogenetice and molecular biological diagnostic tests, invention of a cord blood bank and develop the research activities in these fields.

I-77: Functional Genomics of Embryonic Stem Cells and Derived Mesodermal Lineages

Hescheler J

University of Cologne,Institute of Neurophsiology, Cologne, Germany
Email: J.Hescheler@uni-koeln.de

I-78: Force Measurements of Human Embryonic Stem Cell-Derived Cardiomyocytes in an In Vitro Transplantation Model

Hescheler J

University of Cologne,Institute of Neurophsiology, Cologne, Germany
Email: J.Hescheler@uni-koeln.de

Objective: Human embryonic stem cell (hESC)-derived cardiomyocytes have been suggested for cardiac cell replacement therapy. However, there are no data on loaded contractions developed by these cells and the regulation thereof.
Materials and Methods: We developed a novel in vitro transplantation model in which beating cardiomyocytes derived from hESCs (line H1) were isolated and transplanted onto noncontractile, ischemically damaged ventricular slices of murine hearts.
Results: After 2–3 days, transplanted cells started to integrate mechanically into the existing matrix, resulting in spontaneous movements of the whole preparation. Preparations showed a length-dependent increase of active tension. In transplanted early beating hESC-derived cardiomyocytes, frequency modulation by field stimulation was limited to a small range around their spontaneous beating rate. .
Conclusion: Our data demonstrate that this novel in vitro transplantation model is well suited to assess the mechanical properties and functional integration of cells suggested for cardiac replacement strategies

Keywords: Pluripotent Stem Cells, Myocardial Contraction, Cell Transplantation, Myocardial Infarction, Transplants

I-79: Reprogramming of Adult Cells into Embryonic Cells

Hochedlinger K

Center for Regenerative Medicine, and Cancer Center, Massachusetts General Hospital, 185 Cambridge Street, CPZN 4242, Boston, MA 02114, USA
Email: khochedlinger@helix.mgh.harvard.edu

Ectopic expression of the four transcription factors Oct4, Sox2, c-Myc, and Klf4 is sufficient to confer a pluripotent state upon the fibroblast genome, generating induced pluripotent stem (iPS) cells. It remains unknown if nuclear reprogramming induced by these four factors can globally reset the epigenetic differences between differentiated cells and pluripotent embryonic cells. Here, using novel selection approaches, we have generated iPS cells from fibroblasts to characterize their epigenetic state. Like ES cells, female iPS cells showed reactivation of a somatically silenced X chromosome and underwent random X inactivation upon differentiation. Moreover, genome-wide analysis of two key histone modifications, H3K4 and H3K27 trimethylation, indicated that iPS cells are highly similar to ES cells. Consistent with these observations, iPS cells gave rise to viable high degree chimeras with contribution to the female germ line. Together, these data show that transcription factor-induced reprogramming leads to the global reversion of the somatic cell epigenome into an ES-like state. Our results provide a paradigm for studying the epigenetic modifications that accompany nuclear reprogramming and suggest that abnormal epigenetic reprogramming likely does not pose a problem for the potential therapeutic applications of iPS cells.

I-80: Embryonic Stem Cells and Pluripotency

Hochedlinger K

Center for Regenerative Medicine, and Cancer Center, Massachusetts General Hospital, 185 Cambridge Street, CPZN

4242, Boston, MA 02114, USA
Email: khochedlinger@helix.mgh.harvard.edu

Mammalian development has been thought to be a one-way process, which starts with a few embryonic founder cells, that become more and more restricted and ultimately give rise to all specialized cell types of the body. The cloning of the sheep Dolly from an adult mammary gland cell has refuted this dogma and demonstrated that the developmental clock of a mature cell can be reset, or "reprogrammed" by the egg into that of an embryonic cell, which can support development of a copy of the donor animal. To exclude the possibility that adult stem cells served as donors in successful cloning experiments, we have generated cloned mice from mature lymphocytes that carried immunoglobulin rearrangements in all tissues. This experiment demonstrated that even the nuclei of terminally differentiated adult cells remain competent to give rise to an entire cloned animal. In addition, reprogramming research has enormous therapeutic potential in humans as it may allow for the derivation of embryonic cells from patients' cells suffering from degenerative disorders such as Alzheimer's disease, Parkinson's disease or diabetes; because embryonic cells have the ability to give rise to all cell types of the body when exposed to the right combination of growth factors, these cells may provide a unique source of replacement tissue for regenerative medicine. We have generated a mouse model to prove this concept by combining nuclear transfer with gene and cell therapy to treat a severe combined immunodeficiency disorder in mice.

I-81: Testis Tissue Xenografting-A Novel In Vivo Culture System to Study Testis Function

Honaramooz A

Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5B4
Email: ali.honaramooz@usask.ca

Spermatogenesis is inherently a highly organized, efficient and complex process controlled by cellular and hormonal interactions that are not well understood. In order to comprehensively investigate testis function, an in vitro or in vivo model for spermatogenesis is required that is accessible and can faithfully mimic the donor tissues environment. Testis tissue xenografting involves grafting of small fragments of testis tissue from immature donor males of a variety of mammalian species under the back skin of host mice to allow the grafted tissue to grow and develop. Testis tissue xenografting has numerous potential applications. Perhaps most importantly, it can be used as an in vivo culture system to study testis function from a variety of donor species in a laboratory mouse. Using this culture system, virtually all aspects of testis function remain intact and the testis tissue is accessible for study and interventions. Therefore, it can serve as a unique and previously unavailable in vivo system for the study of spermatogenesis and steroidogenesis in a controlled manner not feasible in the target donor species. Completion of primate spermatogenesis in testis tissue fragments originating from prepubertal donor monkeys grafted into immunodeficient mice shows this technique can be used to examine spermatogenesis of primates and possibly humans, and can potentially be used as an in vivo system to study the endocrine/paracrine control of human testis in a laboratory model. This for example can be used to test the in vivo effects of new male contraceptives in the form of candidate compounds or promising hormone-therapy regimens directly on miniature primate testes incubated inside a mouse.

I-82: Lessons Learned from Transplantation of Spermatogonial Stem Cells

Honaramooz A

Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5B4
Email: ali.honaramooz@usask.ca

Spermatogonial stem cells (SSCs) form the basis of spermatogenesis resulting in the production of countless numbers of sperm throughout adulthood in a male. In addition to being extremely efficient, SSCs are unique in that they are the only stem cells in an adult body that can contribute genes to the next generation. These attributes point to the tremendous potential of SSCs as a source of genetic change in the progeny. Our knowledge of SSCs, however, began to dramatically increase only after a system for transplantation of these cells was developed by Ralph Brinster from the University of Pennsylvania in 1994. In this transplantation system, dissociated testis cells from a donor male are microinjected into the seminiferous tubules of the recipient testis. While the population of testicular donor cells may contain a heterogeneous mixture of different types of germ cells and somatic cells, only true spermatogonial stem cells can colonize and initiate new spermatogenesis in the recipient testis, making this transplantation system a functional assay for unequivocal detection of SSCs in a given population of testis cells. The process involves a selection step in which Sertoli cells recognize and allow SSCs to migrate from the lumen of the seminiferous tubule, where they are deposited, to the basal membrane which contains the stem cell niche. Ever since its advent, this transplantation system has played an instrumental role in hundreds of studies, greatly expanding the field of male reproductive biology and shaping up our current understanding of SSCs. This includes learning some crucial facts about SSCs, such as their ability to maintain developmental potential after long-term culture, cryopreservation, and genetic modification. This system not only has made it possible to study markers for SSCs but also for their continued self-renewal. We have also learned that: SSCs' characteristics are conserved among closely-related spe-

cies; SSCs have the ability resist a number of cytotoxic insults; can also be genetically modified; forced into proliferation in vitro; and made to differentiate into a number of cell lineages. These lessons have broaden our general knowledge of adult stem cells and provided potential alternatives to overcome some forms of male infertility.

I-83: Comparative Proteome and Transcriptome Analyses of Embryonic Stem Cells during Embryoid Body-based Differentiation

Hosseini Salekdeh Gh[1,2*], Baharvand H[1,3], Fathi A[1], Nasrabadi D[1], Taei A[1], Adjaye J[4], Pakzad M[1], Shekari F[1], Pirhaji L[1], Rezaei M[1], Mollamohammadi S[1]

1. Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran
2. Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
4. Department of Vertebrate Genomics, Max-Planck Institute for Molecular Genetics, Berlin, Germany
Email: hsalekdeh@yahoo.com

Gene expression analyses of the embryonic stem cells (ESCs) will provide insights into signaling pathways and molecular mechanisms involved in the maintenance of self-renewal and pluripotency. Transcriptomics and Proteomics approaches proved to be powerful approaches to identify many components differentially regulated during ESC proliferation and differentiation. We analyzed proteome of differentiated and undifferentiated human, mouse and monkey ESCs. Comparative analyses of differentially expressed proteins revealed several proteins as key participants in stem cell proliferation and differentiation. The trancriptome of proliferating and differentiating human ESCs was analyzed using a microarray approach. Our results showed that proteomics and transcriptomics data are complementary rather than duplicative. Although regulation of many genes during differentiation were observed only at transcript level, modulation of several proteins involved in cell growth and cell cycle, transcription regulation, signal transduction, transcription, and translation was revealed only by proteome analysis.

I-84: Biodegradable Nanoparticles for Long Term Tracking of Stem Cells for Biological Applications

Hosseinkhani H

BioCells, Inc., Shibuya-ku, Tokyo 162-8656, JAPAN
Email: hossein@mit.edu

Human embryonic stem (ES) cells have great potential for use in biological and medical applications because of their remarkable ability to differentiate into all cell types of an adult, including cardiomyocytes. The clinical use of human ES cells has raised considerable ethical issues which have been a topic of much discussion. The use of markers for labeling cells is critical to tracking transplanted cells in the body. To track ES cells in vivo, cells should be labeled with an imaging contrast agent that is non-toxic and does not alter the differentiation of ES cells prior to implantation. Recently, magnetic labeling of transplanted mammalian cells has been used to monitor the location of cells in a non-invasive manner. Cells containing a magnetic label can be tracked non-invasively in living tissues using magnetic resonance imaging (MRI). To enable this technology for ES cells it is important to transfect the cells with labeling agents prior to transplantation in a manner that is efficient and does not alter cell behavior. The long term tracking of ES cells is particularly useful for tissue engineering applications such as cardiac regeneration. Magnetic labeling ES cells could be a powerful tool to study their differentiation process into different type of cells such as cardiomyocytes. To enable the non-invasive monitoring of ES cells derived differentiated cells, we have developed very stable magnetic biodegradable nanoparticles to label different type of stem cells such as ES cells prior to their differentiation such as cardiac differentiation. In the present work, I will introduce to you one of our recent technologies currently available for stem cells marketing applicable for regenerative medicine.

I-85: Towards the Development of Advanced Medicine by Use of Nanotechnology

Hosseinkhani H

BioCells, Inc., Shibuya-ku, Tokyo 162-8656, JAPAN
Email: hossein@mit.edu

As the third surgical therapy following the reconstruction surgery and organ transplantation, a new therapeutic trial based on the natural potential of tissue regeneration induction has been expected. For successful tissue regeneration, it is necessary to make use of cells, the scaffold of cell proliferation and differentiation, and growth factor or their combination. With recent research advance of basic biology and medicine of cells, various stem and precursor cells of high proliferation and differentiation potentials have been available experimentally and clinically. However, only by using such cells, it is practically difficult to induce tissue regeneration. This is because basically, cells survive and biologically function interacting with their local surrounding environment which has been demonstrated to be comprised from growth factors and extracellular matrix. Various biomaterials and the related technology or methodology have been used to create an artificial environment which enables cells to induce tissue regeneration. If cells around a tissue defect have an inherent regeneration ability, tissue regeneration will be induced only by supplying a temporary cell scaffold of biomaterials to the defect. However, this approach is ineffective for the site of poor regeneration potential. In this case, the combination with cells and/or growth factors is required. It is well recognized that a variety of growth factors act on cells forming complex networks to

build the local regeneration environment. If a key growth factor is supplied to the right place at the right time period and concentration, it is no doubt that the body system will initiate to function, resulting in natural induction of tissue regeneration. However, only when the growth factor of in vivo instability is injected in the solution form, the in vivo biological activities cannot be always expected. One practically possible way to enhance the in vivo activities expected is to make use of drug delivery system (DDS). We have prepared biodegradable nanoparticles for the controlled release of bioactive growth factors and plasmid DNA to demonstrate the successful regeneration repairing of various tissues. This release system can be combined with cells and/or the cell scaffold to induce the regeneration repairing of tissues and organs. In the present lecture, I will introduce to you several experimental data of tissue regeneration on the basis of nano-biomaterials and DDS technology.

I-86: Microengineered Hydrogels for Tissue Engineering

Khadem Hosseini A

Harvard-MIT, Division of Health Sciences and Technology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA
Email: alik@rics.bwh.harvard.edu

I-87: Microengineered Systems for Directing Stem Cell Differentiation

Khademhosseini A

Harvard-MIT, Division of Health Sciences and Technology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA
Email: alik@rics.bwh.harvard.edu

I-88: Mesenchymal Stem Cells for Treatment of Acute GVHD

Le Blanc K

Hematology Center, Division of Clinical Immunology, Karolinska University Hospital Huddinge
141 86 Stockholm, Sweden
Email: katarina.leblanc@ki.se

I-89: Hematopoietic Stem Cell Transplants

Le Blanc K

Hematology Center, Division of Clinical Immunology, Karolinska University Hospital Huddinge
141 86 Stockholm, Sweden
Email: katarina.leblanc@ki.se

I-90: Generation of iPS Cell from Adult Cells and Potential Applications of iPS Cell Technology

Liao J, Wu Z, Xiao L*

1. Laboratory of Molecular Cell Biology, Key Laboratory of Stem Cell Biology, Institute of Biochemistry and Cell Biology, Cell Bank, Stem Cell bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, P.R. China
E-mail: leixiao@sibs.ac.cn

Murine and human fibroblasts have been reprogrammed to the undifferentiated state by ectopic expression of defined transcription factors(Oct4, Sox2, Klf4, Myc) and (Oct4, Nanog, Sox2, Lin28). Using the combination of the 6 transcriptional factors, Oct4, Nanog, Sox2, Lin28, c-myc and Klf4, we have derived iPS cells from human newborn foreskin fibroblasts. These human induced pluripotent stem cells express alkaline phosphatase and the undifferentiated human embryonic stem cell-specific cell surface antigens, have the ability to differentiate into all three germ layers in embryonic bodies and are more efficiently and rapidly generated than the 4 factors induced ones. Our protocol should facilitate the study of the mechanism of somatic cell reprogramming, the generation of patient-specific stem cell lines to study different disease mechanisms and eventual transplantation therapies.

Keywords: Human Embryonic Stem Cell, Pluripotency, Reprogram, iPS

I-91: DNA Methylation in Early Embryogenesis

Nakano T

Department of Pathology, Medical Schools, Osaka University, Osaka, Japan
Email: tnakano@patho.med.osaka-u.ac.jp

DNA methylation is one of the important modifications in epigenetic gene regulation, and the control of DNA methylation status is a prerequisite for normal early embryogenesis and cell differeititaion. We have been analyzing two proteins, PGC7/Stella and MILI, which play crucial roles in the DNA methylation in early embryogenesis and spermatogenesis, respectively.
Although global demethylation occurs soon after fertilization, demethylation does not take place on the whole genome evenly. Maintenance of the methylation in the imprinted genes and epigenetic asymmetry between parental genomes, i.e., delayed demethylation of the maternal genome after fertilization are good examples of the protection of DNA methylation status. As one of the topics, I will show that PGC7/Stella, a maternal factor essential for early development, plays some roles in the protection of the DNA methylation in several genomic imprinting loci and epigenetic asymmetry. After determining that PGC7/Stella binds to Ran binding protein 5 (RanBP5), a nuclear shuttle transporter protein, we examined the exact time when, and subcellular location where, PGC7/Stella functions, using mutants of PGC7/Stella and RanBP5. PGC7/Stella turns out to be implicated in protecting the maternal genome from demethylation only after localization to the nucleus and maintaining the methylation of several im-

printed genes. These results demonstrate that PGC7/Stella is an indispensable methylation protector involved in epigenetic reprogramming after fertilization.
The other topic I will introduce is the function of small RNA in gene silencing. Gene silencing in mammals is believed to be controlled by RNA interference in the same way as in other organisms; however, the molecules involved in the process remain unclear. The Argonaute proteins which include Piwi family proteins, are the candidates for controlling the silencing process. We have shown that a member of mouse Piwi family, MILI, plays crucial roles in the piRNA class of small RNA processing and/or protection, and subsequent gene silencing of retrotransposons through de novo DNA methylation at early spermatogenesis.

I-92: PI3K/Akt Signaling in Germ Cell Development

Nakano T

Department of Pathology, Medical Schools, Osaka University, Osaka, Japan
Email: tnakano@patho.med.osaka-u.ac.jp

Stem cells can replenish their own population while supplying the cells necessary to maintain tissue homeostasis. Pluripotent stem cells, which have broader developmental potency than tissue stem cells, are derived from the same source in mice and humans. We have been analyzing the functions of phosophoinositide-3 kinase (PI3K) and its downstream serine/threonine kinase Akt in a variety of stem cell systems.
Primordial germ cells (PGCs), which are embryonic germ cell precursors, are unique in that they acquire pluripotency under cultural and pathological conditions. PGCs lacking Pten, which encodes a phosphatase that antagonizes PI3K signaling, give rise to early-onset testicular teratomas in vivo and augment the derivation of pluripotent embryonic germ (EG) cells in vitro. Transient activation of Akt sufficiently recapitulates the effects of Pten deficiency on EG cell derivation. Enhanced EG cell derivation is brought about by the Akt-mediated inhibition of the tumor suppressor p53. In embryonic stem (ES) cells, PI3K/Akt signaling plays a pivotal role in maintaining pluripotency in part via transcriptional activation of the pluripotent transcription factor Nanog. In turn, the expression of Tcl1, a cofactor of Akt, is activated by pluripotent transcription factors, including Oct-3/4. Therefore, PI3K/Akt signaling and the transcription factor network constitute the positive feedback circuitry necessary to maintain pluripotency in ES cells.
In tissue stem cells, such as hair follicular, intestinal, and hematopoietic stem cells, PI3K/Akt signaling activates quiescent stem cells, leading to the generation of committed progenitors and cancer stem cells. These findings underscore the idea that PI3K/Akt signaling regulates "stemness" in many stem cell systems.

I-93: In Vitro Recapitulation of Stem Cell Niche

Nishikawa S

Riken Center for Developmental Biology, Japan
Email: nishikawa@cdb.riken.jp

Stem cell (SC) locates at the top of the hierarchy of SC system to replenish all cells including themselves. Quiescence is an important features distinguishing SC from other compartments for most SC systems. A line of evidence suggests that this quiescent state is directed by external cue expressed in the special microenvironment for SC, which is designated as stem cell niche, though its molecular nature remains unclear for most stem cell systems. Our group has been addressing this question using melanocyte (MC) as a model. In mouse, MC SC was able to be defined by its specific localization in the bulge region of hair follicles, whereas other compartments are located in the hair matrix at the bottom of hair follicle (Nishimura et al 2002). By virtue of previous studies to characterize MC SC, we are now able to define MC SC by a combination of gene expression pattern and functional properties (Osawa et al,2005). Moreover, we showed that MC SC is induced during postnatal few days (Mak et al 2006). Thus, next important question is to specify extrinsic molecules that direct the fate of proliferating embryonic melanoblasts (MB) to quiescent SC. One approach to this issue is to mutate SC specific genes one by one to determine their involvement. As MC per se is not essential for the life and its defect is easily detected as coat-color changes, MC is an ideal lineage for applying methods of conditional gene manipulations. Indeed, we determined the role of Notch 1 in SC by this manner (Moriyama et al. 2006). However, we think that KO approach may not be efficient to determine the molecules that are involved in the induction process of quiescent SC from embryonic MC. On the other hand, this question could be more properly addressed in the culture system where the activity of MB are recapitulated. Such a culture system should be useful to evaluate molecules that induce transition of MB to quiescent MC SC. With this rationale in mind, we have established a culture system for MB purified from embryos (Yonetani et al 2007) and have used this culture for inducing quiescent SC.
In this symposium, we will describe our results on the process of transition from MB to MC SC, which will show that induction of SC is a complex step requiring multiple steps. This induction process can be monitored by 4 distinct properties of MC SC; 1) survival in the absence of extrinsic signals including SCF, 2) downregulation of melanocyte specific genes, presumably due to deprivation of Wnt signal, 2) global suppression of transcription due to inhibition of serin 2 phosphorylation of RNA polymerase, and 4) nuclear localization of Foxo proteins. The culture system was used to search for the molecules that can induce those SC properties. The first molecule came out from our studies was FGF that is able to induce some of above SC features. However, further work is needed to induce all the four features in vitro. In the symposium, the latest result will be presented and discussed.

I-94: Dissecting Differentiation Pathway of Hematopoietic Stem Cells In Vivo and In Vitro

Nishikawa S.*, Samokhvalov I

Riken Center for Developmental Biology, Japan
Email: nishikawa@cdb.riken.jp

A goal of our research is to induce the definitive hematopoietic stem cell (dHSC) in vitro from plurpotent cell lines. As ES cells and iPS as well are the sole cell line that can be maintained in vitro and is proven to give rise to dHSC, this is the most promising culture for producing a unlimited number of dHSC. At this moment, however, this is a difficult goal to attain We think that this difficulty is due to the lack of our knowledge about the actual differentiation process to dHPC in the embryo. Previous studies of embryonic HSC differentiation focused on either the molecules required for HSC differentiation or on the site where the first dHSC are generated. Studies in this line indeed facilitated our understanding on key players such as Runx1 that is involved in this process. On the other hand, only a little is known about the actual pathway for the dHSC specification nor about the phenotype of dHSC progenitors. Indeed, even the notion of hemangioblast is tottering. Thus, what to be clarified first should be the actual differentiation pathway of the dHSC, without which one can not specify the cells to be generated in the culture.

We are addressing this issue by genetic approaches. One approach is to label the progenitors of dHSC at a given time point by the tamoxifen-inducible Cre-recombinase mediated activation of LacZ gene. The second approach also uses the tamoxifen-inducible Cre-recombinase, but this time Cre was used to reactivate runx1 gene in runx1-/- mouse. By these approaches, we specified that E7.5 Runx1+ cells in the YS are the progenitors of dHSC. While dHSC have been suggested to be generated from multiple sources in the embryo, our results suggest strongly that all of them are derived from this Runx1+ cells in the YS. Thus, the new scheme came out from our studies is completely distinct from what has been believed as consensus concerning the differentiation of dHSC.

Once the actual pathway of dHSC is defined, it is possible to address whether or not this Runx1+ progenitors are induced from ES cells. Indeed, the population that is almost equivalent to this progenitors are able to be generated in ES cell culture. In addition, it should be noted that Runx1+ cells in E7.5 YS are unable to generate dHSC in vitro. Hence, it is likely that the failure of ES cell culture to generate the dHSC is not due to deficiency of ES cell culture in inducing the dHSC progenitors, but due to deficiency to support further differentiation of the Runx1+ progenitors to the stage that can reconstitute the irradiated recipient. Currently, we are trying to identify the molecular basis underlying this deficiency. In the symposium, we will present the latest results of this attempt.

I-95: Combinatorial Signals of Activin/Nodal and Bone Morphogenic Protein Regulate the Early Lineage Segregation of Human Embryonic Stem Cells

Wu Z[1,4], Zhang W[1,2,4], Chen G[3], Cheng L[1], Liao J[1], Jia N[1], Gao Y[1], Dai H[1], Yuan J[2], Cheng L[3], Xiao L[1*]

1. Laboratory of Molecular Cell Biology, Key Laboratory of Stem Cell Biology, Institute of Biochemistry and Cell Biology, the Cell Bank/Stem Cell bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, P.R. China
2. Department of Biotechnology, College of Life Science, Shandong Normal University, Jinan, 250014, P.R. China
3. Stem Cell Program, Institute for Cell Engineering, and Department of Gynecology & Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 4. Zhao Wu and Wei Zhang contributed equally to this work
E-mail: leixiao@sibs.ac.cn

Cell fate commitment of pre-implantation blastocysts, to either the inner cell mass or trophoblast, is the first step in cell lineage segregation of the developing human embryo. However, the intercellular signals that control fate determination of these cells remain obscure. Human embryonic stem cells (hESCs) provide a unique model for studying human early embryonic development. We have previously shown that Activin/Nodal signaling contributes to maintaining pluripotency of hESCs, which are derivatives of the inner cell mass. Here we further demonstrate that the inhibition of Activin/Nodal signaling results in the loss of hESC pluripotency and trophoblast differentiation, similar to BMP4 induced trophoblast differentiation from hESCs. We also show that the trophoblast-induction effect of BMP4 correlates with and depends on the inhibition of Activin/Nodal signaling. However, the activation of BMP signaling is still required for the trophoblast differentiation when Activin/Nodal signaling is inhibited. These data reveal that the early lineage segregation of hESCs is determined by the combinatorial signals of Activin/Nodal and BMP.

Oral Presentations

Andrology

O-1: The Antioxidantal Effect of Pomegranate Juice on Sperm Parameters and Fertility Potential in Mice

Amini rad O

Yazd Medical University, Yazd, Iran
Email: omid_amini2007@yahoo.com

Objective: This study was an attempt to explore the antioxidantal effect of pomegranate juice on sperm parameters (count, morphology, motility) and fertility potential in mice.
Materials and Methods: 15 male mice were studied with regard to their sperm parameters and fertility potential. Sperms were categorized into three groups with regards to their motility: progressive, non-progressive, immotile. Morphology consisted of normal and abnormal sperms. Mice were divided into control group (n=5) and experimental group (n=10). The experimental group received 20% pomegranate juice for 1 month (duration of spermatogenesis is 1 month in mice). The control group had free access to water. We took one generation from each group to study the fertility rate. After killing the animals, a sample from the tail of epididymal region was taken to test the sperm parameters by light microscope.
Results: The results showed that motility and count of sperms didn't change significantly in both groups. However, the normal morphology and the fertility potential of the experimental group improved significantly. Normal morphology in control group was 68.8±4.76%, and in experimental group was 79.1±6.26% (p=0.007). The rate of fertility in control group was 5.8±4.08 and in experimental group was 10 ±1.26 (p=0.04). Also the rate of progressive sperms in control group was 35.6 ±9.91%; while, in experimental group increased to 47.5 ±11.10% (p=0.063).
Conclusion: The pomegranate juice is an effective antioxidant that is able to improve the quality of sperm parameters, especially sperm morphology, as well as fertility potential in mice. Probably, intake of this antioxidant by infertile men improves the quality of their sperm parameters.

Keywords: Pomegranate Juice, Antioxidant, Sperm, Fertility Potential, Mouse

O-2: The Effect of Zinc Administration on Spermatozoal Protamine Deficiency Induced by Local Heating of Mice Testis

Azizollahi S*, Babaei H

Veterinary Department, Shaheed Bahonar University, Kerman, Iran
Email: saeedazizollahi@yahoo.com

Objective: Protamines are testis-specific nuclear proteins that facilitate chromatin condensation and compaction. Any factors causing an increase in testicular temperature can alter sperm chromatin structure associated with decrease in protamine result in male infertility. In this study, we investigated the effect of zinc therapy on sperm chromatin structure changes as a result of testicular heating.
Materials and Methods: Forty-eight NMRI mice were divided randomly into 4 equal groups each containing twelve animals. Mice in groups 1 (HS, heat saline) and 2 (HZ, heat zinc) were anaesthetized and their scrotums were immersed for 15 min in a water bath maintained at 43 °C. Animals in groups 3 (CS, control saline) and 4 (CZ, control zinc) were treated identically except that the water bath was maintained at 23 °C. Just after this, mice in groups 2 (HZ) and 4 (CZ) were given 10 mg/kg zinc sulphate intrapritoneally, and this treatment was continued every other day for 60 days. Animals in groups HS and CS were given sterile saline instead of zinc. An additional experimental group was also used as a sham group (n=4). Four mice were scarified in each experimental group at 15, 30 and 60 days after treatment and the cauda epididymis were quickly excised. Protamine deficiency was assessed by Chromomycin A3 (CMA3) staining following sperm sample preparation.
Results: After 15 days, heat treatment reduced the proportion of sperms with sufficient protamine in the HS group in comparison to the CS group (90.1±0.65 vs. 99.6±0.23, $p<0.001$). In the course of examination, protamine deficiency was improved in the HS group whether was still significantly lower than that of the CS group at the day 60 (98.2±0.33 vs. 99.5±0.20, $p<0.05$). Administration of zinc did not alter the proportion of sperm nuclear protamine by itself. However, sperms in the HZ group did not have nuclear protamine deficiency ($p>0.05$).
Conclusion: As a consequence, after local heating of mice testis, immediate administration of zinc especially during the first 15 days may prevent the progression of deprotamination of sperm nuclei.

Keywords: Protamin, Zink, Heat

O-3: The Effects of Nitric Oxide Synthase Inhibitor (L-NAME) on Epididimal Sperm Count, Motility, Morphology and In Vitro Fertilization Capacity of Varicocelized Rat

Bahmanzade M[1*], Amiri I[1], Alizadeh Z[1], Abbasi M[2], Abolhassani F[2]

1. Anatomy Department, Hamedan University, Hamedan, Iran
2. Anatomy Department, Tehran university, Tehran, Iran
Email: bahmanzadeh12@yahoo.com

Objective: Nitric oxide has been reported to be increased in the spermatic veins of men affected by varicocele. Although several authors have considered the relationship

between varicocele and semen NO concentrations, but no one studied about the effects of nitric oxide synthase inhibitor (L-NAME) on in vitro fertilization capacity of Varicocelized Rat. Authors have believed that a partial obstruction of the spermatic vein is the only procedure able to induce a varicocele similar to that happen in human being.
Materials and Methods: Twenty four Wistar male rats divided into four groups. The group A and B underwent a left experimental varicocele (we used 20-gauge needle). Group C, underwent a similar procedure to groups A and B without any changing on spermatic vein (as sham group). Group D referred to as control. Animals in group A were killed 8 weeks after the operation and both left and right Epididymal sperm parameters and in vitro fertilization capacity were evaluated. At this time the animals in group B were received intraperitoneally 10mg/kg L-NAME daily for 8 weeks
Results: In group A, Sperm count and morphology were significantly decreased in comparison with the group C and D. The sperm morphology and count between the groups A and B had showed statistically significant differences ($p<0.0001$). Sperm motility and in vitro fertilization capacity decreased significantly in the group A in comparison with the group C and D. However, in group A , motility and in vitro fertilization capacity showed differences in comparison with group B but there were not statistically significant.
Conclusion: These findings suggest that nitric oxide synthase inhibitor (L-NAME) improved statistically sperm count and morphology and improved motility in varicocelized rat.

Keywords: Nitric Oxide Synthase Inhibitor, Sperm Parameters, In Vitro Fertilization Capacity, Varicocele, Rat

O-4: Existence of Leptin's Receptor (Ob-R) in Spermatozoa from Infertile Males

Hatami L

Anatomy Department, Cell Sciences Research Center, Royan Institute (Isfahan Compus), ACECR, TIsfahan, Iran
Email: L.hatami1938@gmail.com

Objective: Leptin, a 167 amino acid peptide, is known to influence reproduction. This hormone indirectly via the central neuroendocrine system and directly, via peripheral tissue membrane receptors has impact on the reproduction function. Thus, biological effects of leptin mediate by interacting with leptin receptors. It has been demonstrated that, leptin receptors (Ob-R) have been localized in human spermatozoa and may play a function on fertility. Thus suggesting a possible action of this hormone even on these cells. Our aim of this study to verify leptin receptor (Ob-R) in spermatozoa of infertile males
Materials and Methods: Semen samples were collected from infertile couple referred to fertility and infertility Esfahan center. Semen analysis was carried out according to WHO criteria. For evaluated leptin receptor, we used several techniques such as Immunostaining, Flow cytometry and Reverse transcriptase-polymerase chain reaction (RT-PCR).
Results: Leptin receptor (Ob-R) was not detectable on the spermatozoa of infertile and fertile men with abnormal spermiogram parameters by Immunofluorescence microscopy or neither by Flow cytometry assay. Furthermore information, we assessed leptin receptor expression (Ob-R) by RT-PCR method. That, leptin receptor expression did not observe in spermatozoa or it was very slightly.
Conclusion: Unlike to previous study, the results of this study suggest that Leptin receptor (Ob-R) expression on spermatozoa of infertile male was very low or none detectable. However, one study shows that Leptin receptor present in spermatozoa from fertile man. But, we did not observe this receptor in fertile group. Despite, we used several methods and antibody in this study unlike previous study.

Keywords: Human, Leptin Receptor, Spermatozoa, Infertility

O-5: First Evidence of Disturbed Expression of the Oocyte-Activating Factor PLCζ in Globozoospermic Men

Heytens E[1*], Gerris J[1], Dhont M[1], De Sutter P[1], Parrington J[2], Young C[2], Coward K[2], Lambrecht S[3], Deforce D[3], Cuvelier C[4]

1. Centre Reproductive Medicine, Ghent University Hospital, Gent Belgium
2. Pharmacology Department, University of Oxford, Oxford, UK
3. Pharmaceutical Biotechnology Department, Ghent University, Gent, Belgium
4. Pathology Department, Ghent University Hospital, Gent, Belgium
Email: elke.heytens@ugent.be

Objective: At fertilization the oocyte is activated by a sperm-induced rise in intracellular calcium concentration within the egg. In mammals there is now substantial evidence to suggest that egg activation is induced by a sperm-specific phospholipase C named PLCζ. It is possible that a decreased concentration or an inactive form of the oocyte activating factor in the sperm cell could therefore account for some cases of failed fertilization after intracytoplasmic sperm injection (ICSI). The oocyte activating capacity of spermatozoa from globozoospermic men is severely diminished, as shown by the low or totally absent fertilization after ICSI and by the negative results of the mouse oocyte activation test (MOAT). We therefore set out to test whether PLCζ is absent or inactive in spermatozoa from globozoospermia patients.
Materials and Methods: Fresh or cryopreserved semen samples were provided from four globozoospermic pa-

tients and from four proven fertile men. Semen analysis was performed according to the recommendations of the WHO. MOAT is routinely performed to analyze the oocyte activation capacity of the sperm from patients with failed fertilization after ICSI. The MOAT result is expressed as the % of mouse oocytes successfully activated after injection of the patient's sperm. Immuno-fluorescent confocal microscopy studies of human spermatozoa were carried out using polyclonal anti-peptide antibodies generated against human PLCζ sequences. For Western Blot analysis the soluble protein fraction of the samples was extracted and equal amounts were subjected to 1D SDS-polyacrylamide gel electrophoresis. Immunoblotting was performed using anti-PLCζ primary antibodies and immunoreactive bands were visualized with the enhanced chemiluminescence detection system. Relative average densities of the bands were statistically compared with t-test.
Results: The semen analysis of the globozoospermia patients indicated moderate asthenozoospermia and absence of sperm cells with normal morphology. The sperm samples consisted of 100% spermatozoa with the typical spherical heads. The MOAT revealed a severely diminished oocyte activating capacity for all patients (0 -11%). The immunofluorescence study of fertile sperm showed PLCζ to be particularly localized in the equatorial and postacrosomal region. Spermatozoa from the infertile patients lack an acrosome and no sperm cells with PLCζ positive staining in neither equatorial nor postacrosomal region were detected. Western Blot analysis showed a less intense band at the native molecular weight (Mw, 70kDa) of PLCζ in the samples from the globozoospermic men (mean relative intensity±s.e.m. 0.45±0.11 versus 1.00±0.15, p=0.015). Moreover, an altered pattern was observed in two of globozoospermia samples with an extra, lower Mw band (57kDa) of PLCζ which was not found in the control samples.
Conclusion: The present study shows a decreased expression level of the native PLCζ protein in spermatozoa from globozoospermic men. Furthermore, a lower molecular weight peptide was detected in the round-headed sperm, which may represent a truncated, inactive form of the PLCζ protein. Importantly, this finding emphasizes the role of PLCζ during fertilization and suggests an association between some types of infertility and a defect in PLCζ expression. Additionally, PLCζ analysis may be suggested as a diagnostic test to identify infertile patients with decreased oocyte activating capacity.

Keywords: Globozoospermia, PLCζ, Oocyte Activation

O-6: Efficacy of Individualized Homeopathic Therapy in Oligospermia (Male Infertility)

Joseph S

Homeopathy Department, New Fathima Medical Center, Burdubai, UAE
Email: drsinsen@gmail.com

The cases of male infertility are on rise all over the world. The treatment in other school of medicine is very costly and unaffordable to many patients because most of the insurance companies are not covering infertility treatment. The simple oral homeopathic medication for a certain period of time has shown increase in sperm count and motility. The male infertility patients are treated homeopathically in Germany, France, India, UK etc; There are 3 scientific studies done on this topic. One study is done in University of Heidelberg, Germany. The other two studies are done in India and one by me.
I have done statistical analysis, which shows that the treatment of Oligospermia with individualized homeopathic therapy was very effective.

O-7: The Comparison of Efficiency of Density Gradient Centrifugation and Zeta Methods in Separation of Mature and Normal Spermatozoa by TUNEL, SCD, Acridine Orange and CMA3

Khajavi N*, Razavi Sh, Tavalaee M, Deemeh M, Nasr Esfahani MH

Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
Email: n_khajavi61@yahoo.com

Objective: The aim of this study was to examine the efficiency of Zeta method and Density Gradient Centrifugation method for the selection of normal sperms by TUNEL, Sperm Chromatin Dispersion (SCD), Acridine Orange (AO) and Chromomycin A3 (CMA3).
Materials and Methods: The study was conducted on 63 patients. Semen analysis was carried out according to WHO criteria. Semen samples were divided into three equal portions. One portion was left untreated (control), the second portion was used for Zeta method and the third portion underwent DGC method. Then, all of the portions were evaluated to sperm morphology (Diff Quick staining), protamine deficiency (CMA3) and DNA integrity (SCD, AO and TUNEL). Coeficients of correlation and student t-test were carried out using SPSS and P-value lower than 0.05 was considered significant.
Results: The mean number of sperm abnormality, protamine deficiency and DNA fragmentation in Zeta and DGC methods were significantly decreased as compared to the control group ($p<0.005$). In addition, Density Gradient Centrifugation method was superior to Zeta method in the selection of sperms with normal morphology ($p<0.005$) and Zeta method was superior to DGC method in the selection of sperms with intact DNA ($p<0.005$).
Conclusion: Both Zeta and DGC methods were effective in the selection of sperm with better quality in terms of normal morphology, normal protamine content and DNA integrity. However, Density Gradient Centrifugation method was superior to Zeta method in the selection of sperms with normal morphology and Zeta method was superior to DGC method in the selection of sperms with intact DNA.

Keywords: Zeta Method, Density Gradient Centrifugation Method, Sperm Morphology, Protamine Deficiency, DNA Fragmentation

O-8: Effect of Growth Hormone on Testicular Dysfunction and Apoptosis Induced By Methotrexate in Rats

Khaki A

Islamic Azad University of Tabriz , Tabriz, Iran
Emai: arashkhaki@yahoo.com

Objective: Methotrexate (MTX) is a chemotherapeutic agent that used for the treatment of a variety of tumors and inflammatory diseases. This study was conducted to evaluate the role of growth hormone (GH) on testicular dysfunction and apoptosis induced by MTX in rats.
Materials and Methods: Fifty male Wistar rats were randomly divided into five groups (n=10 each), with one group serving as controls. In the GH group, GH was intra peritoneally (IP) administered at a daily doses of 0.3 mg/kg for 28 consecutive day. In the MTX group, MTX was IP administered at weekly doses of 1 mg/kg for 4 weeks. In the protective group, GH and MTX were IP administered together at above doses for 28 days. In the treatment group, MTX was administered at above doses for 4 weeks and GH administration was started 14 days after MTX administration for last two weeks. However, the control group received vehicle (IP). Five rats from each group were sacrificed at days 14 and 28. Spermatozoa were removed from cauda epididymis and analyzed for sperm motility, concentration and viability. Testis tissues were also removed and prepared for histological evaluation and TUNEL assay for detection of apoptosis. In addition, serum testosterone level was determined by radioimmunoassay on day 14 and 28.
Results: This study was confirmed MTX had destructive effects on testis germinal cells. There was a significant decrease in sperm count, viability and motility in MTX group when compared with control group ($p<0.05$). The number of TUNEL positive apoptotic germ cells (spermatogonia & spermatocytes) per tubule cross-section increased in MTX group when compared to the control group at day 14 and 28 ($p<0.05$). However, there was no significant difference in the number of TUNEL positive apoptotic germ cells between control and others groups. Testosterone level had significant decrease in MTX, protective and treatment groups when compare to control and GH groups at day 14 and 28 ($p<0.05$). GH had recovery effects on testis histology and improve sperm parameters and serum testosterone level ($p<0.05$) as compared with MTX group.
Conclusion: These results suggested that administration of GH improved testicular function damaged by MTX.

Keywords: GH, MTX, Spermatogenesis, Apoptosis, Testis

O-9: Effects of ChlVPP Chemotherapeutic Protocol on the Spermatozoa Fertility Indices of Male Rats

Mohammad Golizad L[1*], Zare S[2], Nejati V[2*], Eyshi Oskooii A[3]

1. Science College, Biology, Urmia, Iran
2. Hematology and Oncology Department, Medical University of Urmia, Urmia, Iran
Email: Lila579@yahoo.com

Objective: Hodgkin's lymphoma is a malignant disease with an incidence of 2.4 per 100,000annum in developed countries and comprises 6% of childhood cancers. MOPP,ABVD,ChlVPP are chemotherapy regimens that based on stage and intensity of HL are used to treatment of patients. Cytotoxicity and gonadotoxicity are important for selection of treatment especially in young Hodgkin's patients.The goal of this study was to determine the effects of ChlVPP on the spermatozoa fertility indices of male rat.
Materials and Methods: This study is an experimental study. 12 male wistar rats were divided into 2 groups. The animals from drug treated group were gavaged on day 1 through 5 of alternately week with Chlorambucil, procarbazin and prednisolone and were given an intraperitoneal injection of vinblastin on day 1 and 4 of alternately week (ChlVPP) for 6 weeks. The rats from control group were gavaged normal saline and etanol and injected normal saline. Males were mated 2 weeks after end of treatment with virgin female rats. Male were anesthetized and testies and epididymes were removed and weighed. The caput-corpus epididymides were frozen in liquid nitrogen and were homogenized. Heads of spermatozoa were counted using a hemocytometer. Spermatozoa from the cauda epididymides were used for computer- assisted sperm analysis (CASA,Wilei color analysis) and morphology analyses. To evaluate morphology of spermatozoa, the suspension from cauda epididymides was smeared and stained with Eosin-Negrosin. Data were analyzed statistically using the Independent t-test or Mann witney U-test, according to their distribution.
Results: Difference of first weight mean of rats in ChlVPP-treated and control groups (206.6 ± 5.5 vs 212.5 ± 7.2) weren't significant ($t=0.63$, $p=0.53$). The ChlVPP-treated rats significantly reduced body weight but control group weight were increased. Testis and epididymis weights ($p<0.05$) and spermatozoa number and live ratio ($t= 3.7$, $p=0.007$) and in ChlVPP-treated rats were significantly less than control group. All of spermatozoal motility parameters were no significantly different between two groups ($p>0.05$) .The percentage of class B , C and D spermatozoa between two group had significant difference($t= 3.7$, $p=0.012$). In ChlVPP-treated group deformity wasn't significantly different with control group. Result of mating showed infertility in all of male and no pregnancy in female rats on ChlVPP group.
Conclusion: In this study spermatozoa numbers were decreased in ChlVPPgroup as oligospermia . Comparison of

results contrary to previous studies showed that ChlVPP regimen influence less side effects than ABVD on reproductive system on animal model. Infertility was observed in ChlVPP-treated group. We suggest more studies for comparison of ABVD and ChlVPP protocols effect on fertility.

Keywords: Hodgkin Lymphoma, ChlVPP, Infertility, Rat

O-10: Leuprolide Protects Mouse Spermatogenic Cells from Apoptosis Induced by Busulfan

Mohamadghasemi F[1*], Soleimani Rad J[2], Nasiri E[1], Faghani M[1]

1. Gilan Medical University, Anatomy Department, Rasht, Iran
2. Tabriz, Drug Applied Research Center, Tabriz, Iran
3. Gilan Medical University, Anatomy, Rasht, Iran
Email: Parsahistolab@gmail.com

Objective: Chemothearpeutic drugs have adverse effect on male fertility and induce apoptosis in male germ cells. It has been proposed that gonadotropin releasing hormone (GnRH) analogs administered after testicular damage stimulate the recovery of spermatogenesis. This study was performed to investigate whether administration of leuprolide as a GnRH analog could protect spermatogenic cells from apoptosis induced by busulfan in adult NMRI mouse.
Materials and Methods: Adult mice were divided into 4 groups: (A) a control group given DMSO, (B)a group given busulfan intraperitoneal injection, 40 mg/kg at a single dose. (C) a group given leuprorelin subcutaneous injection 7.6 mg/kg. (D) a group given leuprorelin 7.6 mg/kg, 24 hours after busulfan. Evaluation were made histologicaly, determining Johnsen's score and TUNEL assay.
Results: In the group given busulfan, Johnsen's score were 3.81±0.42 and percent of apoptotic germ cells were 33.3±3.06. However in the group given both leuprorelin and busulfan , Johnsen's score were 7.12±0.58 ($p<0.001$) and percent of apoptotic cells were 20.06±3.32 ($p<0.001$).
Conclusion: Leuprorelin protects germinal epithelium during chemotherapy by busulfan partly through reduction of apoptosis in germ cells.

Keywords: Leuprorelin, Spermatogenesis Protection, Apoptosis, Mouse Testis, Busulfan

O-11: Relationship Between Expression Levels of HSPA2 Gene and Chromatin Structure in Patients with Varicocele

Nasr Esfahani MH, Mirhosseini Z*

Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
Email: mirhosseini5@gmail.com

O**bjective:** Heat-shock protein A2 (HspA2) is correlated with sperm maturity, function and fertility, and a dysfunctional expression of such a gene results in abnormal spermatogenesis. The purpose of this study was to compare HspA2 gene expression in spermatozoa from varicocele men and normozoospermic controls. In addition, we evaluated correlation between expression levels of HSPA2 gene and protamine deficiency, DNA fragmentation in patients with varicocele and normozoospermic.
Materials and Methods: Semen samples were obtained from 42 patients with varicocele and 15 normozoospermic. Sperm morphology, motility and concentration were assessed according to World Health Organization criteria. Sperm DNA fragmentation and protamine deficiency evaluated by Sperm Chromatin Dispersion (SCD) test and chromomycinA3 (CMA3) staining, respectively. Expression levels of HSPA2 gene evaluated by RT-PCR.
Results: We found a significant relationship between expression levels of HSPA2 gene and DNA fragmentation in patients with varicocele ($p<0.05$), but there was not any significant relationship between expression levels of HSPA2 and protamine deficiency.
Conclusion: Results demonstrated that HSPA2 protein protected the sperm chromatin from fragmentation. Accordingly, dysfunctional expression of regulated HSPA2 gene may result in abnormal spermatogenesis. Therefore, these results suggested that low expression of HSPA2 gene could decrease fertility potential in patients with varicocele.

Keywords: Varicocele, DNA Fragmentation, HSPA2, Protamine Deficiency

O-12: Haplotype Analysis of the Estrogen Receptor Alpha Gene in Male Genital and Reproductive Abnormalities

Ogata T*, Fukami F, Wada Y, Yoshida R, Watanabe M

NRICHD, Endocrinology and Metabolism, Tokyo, Japan
Email: tomogata@nch.go.jp

Objective: We have recently suggested that homozygosity for a specific "AGATA" haplotype within a ~50 kb linkage disequilibrium (LD) block of the gene for estrogen receptor alpha may raise the susceptibility to cryptorchidism by enhancing estrogenic effects of environmental endocrine disruptors (EEDs).
Materials and Methods: Haplotype analysis of ESR1 was performed in 328 Japanese subjects, i.e., 70 patients with micropenis (MP), 43 patients with hypospadias (HS), 80 patients with spermatogenic failure (SF), and 135 control males.
Results: The LD block was identified in each of the patient groups and in the control males. The frequency of homozygotes for the specific "AGATA" haplotype was markedly higher in the HS patients ($p=0.0000033$, odds ratio [OR]=11.26) and mildly higher in the MP patients ($p=0.034$, OR=3.64) than in the control males, and the specific haplotype was strongly associated with HS ($p=0.0000022$, OR=11.26) and weakly associated with

MP (p=0.040, OR=3.64) in a recessive mode. There was no significant difference between the SF patients and the control males.
Conclusion: Homozygosity for the specific haplotype may raise the susceptibility to the development of male genital abnormalities in response to estrogenic EEDs.

Keyword: Environmental Endocrine Disruptors, ESR1, Haplotype Analysis, Susceptibility, Undermasculinization

O-13: The Effect of Low Doses of Acetylsalicylic Acid on Sperm Quality

Razaghi S*, Mohamadghasemi F, Khajehjahromi S, Hedayati Omami N

Gilan Medical University, Molecular and Cellular Research Center, Rasht, Iran
Email: Siavash_raz@yahoo.com

Objective: The aim of this study was to investigate effects of low doses of acetylsalicylic acid (ASA) as a nonstroidal anti-inflammatory drug on mouse sperm parameters.
Materials and Methods: Adult male mice were divided in 6 groups. ASA were administered intraperitoneally at doses of 2.5, 5, 10, 20 and 40 mg/kg once daily for 14 days, in groups of A-E respectively. Whereas phosphate buffer solution was given 2ml/kg in control group for the same period. Mice were sacrificed on 15 day and analyzed for sperm quality. Parameters measured were sperm count, abnormal morphology and motility. Motility were scored as fast progressive, slow progressive, shaked and immotile. All data were analyzed statistically using ANOVA test.
Results: Sperm count remained unchanged in all groups except in group E that were significantly reduced ($p<0.004$). Abnormal morphology remained unchanged in all groups. Fast progressive motility were significantly reduced in groups of D and E ($p<0.001$). Slow progressive motility were reduced just in group E ($p<0.001$) and shaking sperms were increased in groups of E and D significantly ($p<0.001$). The percent of immotile sperms in groups of D and E were increased significantly.
Conclusion: Results are well supported by low doses of ASA (of 2.5, 5 and 10 mg/kg) has not adverse effects on sperm parameters. Whereas ASA in higher doses of 20 and 40 mg/kg can reduce sperm motility.

Keywords: Acetylsalicylic Acid, Sperm Parameters, Mouse

O-14: The Effect of Amlodipine on Human Sperm Activity

Sadeghzadeh Oskuei B, Ghanbari A*, Khaki A, Roshangar L

Tabriz Medical University, Anatomy Department, Tabriz, Iran
Email: sadeghzadehbo@gmail.com

Objective: Introduction: Ca^{2+} is a ubiquitous intracellular messenger which encodes information by temporal and spatial patterns of concentration. In spermatozoa, several key functions, including acrosome reaction and motility, are regulated by cytoplasmic Ca^{2+} consentration.
Materials and Methods: Functional importance of sperm [Ca^{2+}] signaling: Because there are few aspects of cell physiology that are not subject to some form of regulation by Ca^{2+}, [Ca^{2+}] I is subject to strict spatio-temporal control, allowing specific Ca^{2+}-sensitive responses that can be activated discretely. In ejaculated spermatozoa, [Ca^{2+}] I regulates motility and hyperactivation, chemotoxis, acrosome reaction and is a key player in the process of capacitation. Pharmacological studies suggest that the major channel in the sperm head plasma membrane responcible for modulating calcium entry and intracellular ionized calcium levels could be either an L-type(a class of high voltage-activated) or a T-type (low voltage-activated) voltage-dependent calcium channel. Patch clamp analysis of calcium currents in immature spermatogenic cells demonstrates the presence of T-type currents.
Results: Ca^{2+} flux at the plasmalemma (ion channels): Voltage-operated Ca^{2+} channels (VOCCs) are a family of transmembrane, channel-forming proteins which show strong structural similarity to each other and to the voltage-operated sodium channels. VOCCs were initially classified on this basis into L,N,P/Q,R and T types. Two general classes of calcium channels have been identified: (I) calcium entry channels, and (II) calcium release channels. The prototype VOCC is composed of four subunits (alpha-1, alpha-2, beta and delta). An important function of VOCCs in sperm is likely to be mediation of the bicarbonate-cAMP signal. Ca^{2+} efflux from intracellular stores produces a signal (gating signal) that opens a store-operated channels (SOC) in the plasma membrane. Through these channels Ca^{2+} ions flow into the cytosol producing a sustained rise in the intracellular Ca^{2+} which is belived to lead to acrosome reaction in mammalian and non-mammalian spermatozo. Recently CatSpers, a novel family of ion channels, expressed exclusively in sperm. Four different subunits have been identified: CatSper1, CatSper2, 3, 4. CatSper expression in the testis is first observed when round spermatids appear during spermatogenesis. In mature cells, CatSper2 protein is localized to the sperm flagellum and CatSper1 to the principal piece of the tail, suggesting that CatSper channels may be involved in regulating sperm motility.
Conclusion: Ca2+ pumps: To date, three types of ATP-utilizing Ca^{2+} pumps have been identified: 1) plasma membrane Ca^{2+} ATPase (PMCA), sarcoplasmic-endoplasmic Ca^{2+} ATPase (SERCA) and the secretory pathway Ca^{2+} ATPase (SPCA). In summary, there is strong evidence to indicate that sperm express both PMCA and SPCA and that these Ca^{2+} pumps play a major role in controlling sperm Ca^{2+} homeostasis. The role for SERCA in mature sperm is more tenuous. Role of L-type VOCC in the acrosome reaction and male infertility: The patients

who are medicated by calcium ion channel blockers, for hypertension control, exhibited a reduced ability to sperm function such as acrosome reaction and mobility. One of the such medicines, is amlodipine. Since, amlodipine is used by young patients and indeed alpha-1 subunit of the L-type VOCC has binding sites for amlodipine and other calcium channel blockers, therefore can affect on sperm activity.

Keywords: Calcium Channel Blockers, Amlodipine, Acrosome Reaction, Sperm Motility

O-15: The Effect of Phoenix Dactylifera Pit Powder on Testosterone Level and Germ Cells in Adult Male Rats

Shariati M*, Sharifi E, Kevah M

Department of Postgraduate Studies, Islamic Azad University, Kazeroun, Iran
Email: mehrdadshariati@hotmail.com

Objective: The pit of Phoenix dactylifera contains the chemical compound such as saturated and unsaturated fatty acids. Saturated fatty acid include stearic and palmitic acid and unsaturated include linoleic and oleic acid, that, these compounds inhibit 5- - reductase enzyme. The present study was carried out with the aim of determining the effect of phoenix dactylifera pit powder on spermatogenesis and testosterone level in adult male rats.
Materials and Methods: In this experimental research 45 male rats wistar strain were divided into 5 group of nine including the control group received nothing, the saline group received an equal volume of normal saline as a solvent and the treatment groups received 0.05 , 0.1 and 0.2 mg/kg B.W the powder of Phoenix dactylifera pit for 21 days orally. The results were analysed through Excell, one- way analysis variance and t-test.
Results: The results showed 0.1 and 0.2 mg/kg of powder increased testosterone level and sperm condense in seminiferous tubules and dihydro testosterone level redu¬ce in comparison to the control and saline groups, while it had no significant effect on serum FSH and LH levels.
Conclusion: According to the research results it can be stated that the powder of phoenix dactylifera pit has probably caused increasing testosterone level and decreasing dihydro testosterone level via inhibiting 5- - reductase enzyme that effect by palmitic, stearic, linoleic and oleic acid of the pit.

Keywords: Pit of Phoenix Dactylifera, Testis, Gonadotropin, Testosterone, Rat

O-16: Ultrastructural Study of Germinal Epithelium in Male Mice Treated with Anticancer Drug and GnRH Antagonist

Soleimani Rad J*, Roshangar L

Tabriz University, Embryology and Histology, Ttabriz, Iran
Email: soleimanirj@yahoo.com

Objective: Male factors, mainly spermatogenesis disorder, is responsible for 20-30% of infertility ocuurs in different societies. One of the known cause of spermatogenesis disorder is chemotherapy in patients with cancer. The side effect of chemoterpic agents may last from 10 years up to the end of the life. Since dividing cells are mainly affected by anticancer drugs, the aim of the present study is to investigate the preventive effect of GnRH antagonist as a suppressor of spermatogonial proliferation, on spermatogenic defect produced by anticancer drug (thiotepa).
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as; control, thiotepa (Tgroup) and thiotepa + cetrorelix, a GnRH antagonist, (T+C group). Thiotepa were injected as ip for 5 days at 2.5 mg/kg doses. In T+C group cetrorelix injection was started one week before thiotepa treatment and continued for 3 more weeks. Since spermatogenic cycle in mice is 35 days, mice in all groups were sacrificed 35 days after thiotepa injection. Testicular specimens were fixed in 2% glutaraldehyde and prepared for EM studies. The thin sections were studied with LEO 906 TEM.
Results: Electron microscopic study revealed that in the T group, in comparison to control group, the basement membrane of the seminiferous tubules were irregular and partly disrupted. Spermatogonial cells were separated from each other by huge spaces and contained several intra cytoplasmic vacuoles. Myoid cells appeared thickened and had dense nuclei.The number of cells with apoptotic features were increased. The organels in the germinal cells were altered as follow`ing: The mithocondria were smaller, vacuolated and broken, mainly in spermatids. The Golgi were dispersed and rER were scattered. All the above alterations are considerably reduced in T+C group. The cells in T+C group were carachteristically similar to those in control group.
Conclusion: According to the results it is concluded that GnRH antagonist administration before cancer treatment could prevent the side effects of anticancer drugs.

Keywords: Anticancer Drug, GnRH Antagonist, Spermatogenesis

O-17: Influence of Sperm Chromatin Anomalies on ART Outcome

Tavalee M*

Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
Email: tavalaee.m@gmail.com

Objective: To evaluate the influence of DNA fragmentation, DNA methylation, and protamine deficiency as

indicators of chromatin status on assisted reproductive technology outcome, and also to assess the relation between these parameters.
Materials and Methods: This study is an experimental research. Semen samples were obtained from 92 couples referred to Isfahan Fertility and Infertility Center for ICSI and IVF treatment. The samples were examined for concentration, morphology and motility according to the WHO guidelines. Semen samples were processed for routine ICSI and IVF using discontinuous Pure Sperm gradients. After insemination of oocytes, the remaining semen samples were used for evaluation of global DNA methylation, protamine deficiency, and DNA fragmentation using immunostaining, Chromomycin A3 (CMA3) and Sperm Chromatin Dispersion (SCD) test, respectively.
Results: Chromomycin A3 staining shows a significant correlation with DNA fragmentation and fertilization rate. Furthermore, unlike in IVF patients, DNA fragmentation showed a significant negative correlation with fertilization rate in ICSI. A significant negative correlation was observed between DNA methylation and DNA fragmentation. In addition, no correlation was found between fertilization rate and DNA methylation in both IVF and ICSI patients.
Conclusion: The results reveal that in ICSI procedure DNA fragmentation and CMA3 affect the fertilization rate, whereas none of these parameters affect post-fertilization development. Furthermore, both CMA3 staining and DNA methylation affect DNA fragmentation, independently of each other. Thus, it can be concluded that these parameters may play an early role in initiation of development.

Keywords: DNA Methylation, Sperm Chromatin Anomalies, ART Outcome

Embryology

O-18: Air Fluid Versus Fluid-Only Models of Embryo Catheter Loading: A Systematic Review and Meta-Analysis

Abou-Setta Ahmed M[1]

5, El-Ashwal Street, from Pyramids Road, Maashal Station, Pyramids, Giza, Egypt
Email: abousetta_md@hotmail.com

Objective: This systematic review was to determine the beneficial or detrimental effect of using air bubbles to bracket the embryo-containing medium during embryo transfer.
Materials and Methods: To test this theory, a meta-analysis of randomized trials comparing air fluid versus fl uid-only methods was performed. The primary outcome measures were live birth, ongoing and clinical pregnancy rates. The secondary outcome measures were the rates of implantation, miscarriage, multiple and ectopic pregnancies and retained embryos.
Results: Conclusion: In conclusion there is insufficient evidence to suggest that the fl uid-only method is superior to the use of air brackets during embryo loading. There is a need for well-designed and powered randomized trials to determine any possible benefit to either method.

Keywords: Embryo Transfer, IVF, Loading of Transfer, Catheters, Meta-Analysis

O-19: Chromosome Stability Differs in Cloned Mouse Embryos and Derivative ES Cells: Implications for Therapeutic Cloning

Sebastian T. Balbach[1*], Anna Jauch[2], Barbara Böhm-Steuer[2], Fatima M. Cavaleri[1], Yong-Mahn Han[3], Michele Boiani[1*]

1. Max Planck Institute for Molecular Biomedicine, Röntgenstraße 20, D-48149 Münster, Germany
2. Institute of Human Genetics, University of Heidelberg, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany
3. Korea Advanced Institute of Science and Technology (KAIST), Department of Biological Science, 373-1 Guseong-dong, Yuseong, Daejeon 305-701, Korea
Email: mboiani@mpi-muenster.mpg.de

Objective: Embryonic stem (ES) obtained by nuclear cloning promise applications in regenerative medicine (therapeutic cloning). So to be, ES cells must be geneticaly and epigenetically normal. Since cloned embryos are frequently doomed, we set out to resolve the contribution of genetic factors to the outcome of cloning, because chromosome aneuploidy may cause gene expression deviations that are indistinguishable from incomplete or faulty epigenetic 'reprogramming'. The specific questions we asked were 1) if the abnormal features of the nuclear transfer (NT) spindle persist or recover with the onset of embryonic divisions, and 2) if such features are passed on to ES cells.
Materials and Methods: Cloned mouse embryos were produced by cumulus NT and derived into ES cells according to our established protocol that has resulted in live cloned mice several times over the years. One of the key features of our cloning protocol is the use ofα-MEM for embryo culture. Control embryos were fertilized in vivo (FD) or produced in vitro by intracytoplasmic sperm injection (ICSI). Spindle analysis was performed at the 4-cell stage after immunostaining for alpha- and gamma-tubulin using a spinning disk laser confocal microscope (Ultraview RS3, Perkin Elmer). Karyotype analysis was also performed at the 4-cell stage after metaphase arrest with colchicine applied at 0.1 μg/ml for 10 hours. Single blastomeres were dissociated from the original 4-cell embryo by zona removal and mild trypsinization before spreading on slides, in order to avoid incidences of overlapping chromosome plates. Untreated embryos that de-

veloped to blastocyst (40%) were either used for ES cell derivation or analyzed for level of DNA methylation at the centromeric satellite (c-satellite) DNA. ES cells were enriched for metaphases by culture in 0.3 µg/ml nocodazole in ES medium for 6 hours. Chromosomes were prepared according to the air-drying method. Slides were stained with Hoechst 33342 (1 µg/mL) and scored on a Nikon TE2000 fluorescence microscope. Only chromosome spreads with ≥40 chromosomes (euploid and hyperaneuploid) were taken into account since spreads with <40 chromosomes are more likely to arise as a preparation artifact. M-FISH analysis was performed for greater chromosome detail. Mouse chromosome specific painting probes were combinatorially labeled using 7 different fluorochromes and hybridized. Metaphase spreads were examined using a Leica DM RXA epifluorescence microscope equipped with a Sensys CCD camera and controlled by the Leica Q FISH software. Image processing and karyotyping were performed using the Leica MCK software. Chromosome spreads with at least one trisomy or structural rearrangement were considered aneuploid.
Results: We found that transplanting mouse cumulus cell nuclei into mouse ooplasts results in structurally abnormal spindles. Contrary to expectations, the karyotype is normal more often in clones than in embryos fertilized via ICSI, although ICSI is not neutral and causes a slight increases of aneuploidy over in vivo fertilization. To gain molecular insight, we reasoned that correct chromosome segregation relies on proper assembly of the kinetochores onto large arrays of tandemly repeated satellite sequences of DNA that are subject to DNA methylation. We found that NT embyos attain the same level of c-satellite DNA methylation as ICSI embryos at the blastocysts stage, despite the hypermethylated status of the cumulus cells. After ES cell derivation, NT embryos lose their edge over fertilized embryos. ES cell lines derived from NT blastocysts have rates of crude aneuploidy (>40/≥40 chromosomes by Hoechst staining) comparable to ES cells derived from FD blastocysts. To expose chromosonal imbalances that may be blind to Hoechst karyotyping, we performed M-FISH analysis of ES cells. M-FISH analysis reveals higher incidence of trisomies and structural rearrangements in NT than in FD ES cells. This might well correspond with higher aneuploidy of the cloned fetus, since ES cells and fetuses share the inner cell mass as a precursor. Cloning only allows a few percent fetuses and newborns, so we were unable to address the matter directly, however we observed a viable and inheritable phenotypic mutation, the first known to be associated with cloning of a mammal.
Conclusion: Our data indicate that defects of the initial NT spindle are not passed on to derivative mitotic spindles during the first three, and the most critical, cell cycles. However, aneuploidy may impinge on later stages, as suggested by the higher rate of chromosomal imbalances found in NT ES cells. These cells are relevant to regenerative medicine (therapeutic cloning). Our findings warn that problems of preimplantation-stage mouse clones are different from those of derivative ES cells, the former being mostly epigenetic, the latter being also genetic. Whether NT ES cells are prone to aneuploidy or not, gene expression levels are kept within the range that is appropriate for the pluripoptent state as long as undifferentiated culture conditions are applied. Therefore, the reported similarity of NT and FD ES cell lines is contingent on selective pressure, and a genuine, inherent similarity is very difficult to prove. Further, our data warn that mutations below the experimental detection limit of the cytogenetic assay are still possible and may unpredictably turn up in cloned animals or derivative cell lines. All mouse clone conditions reported to date had reverted back to normal upon passage through gametogenesis. We show that two cloned embryos from the same experiment developed to term, and that one presented a tail mutation similar to mice heterozygous for a mutation of brachyury (T).

Keywords: Aneuploidy, Embryo, ES Cell, Nuclear Transfer, Spindle

O-20: Comparison Between HA Binding and Zeta Method to Select Mature Sperm for ICSI

Deemeh MR

Cell Sciences Research Center, Andrology Department, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
Email: deemeh77@gmail.com

Objective: At present, sperm selection for ICSI only depends on morphology and motility, but these parameters may not be relevant the chromatin integrity. So sperm selection based on sperm functional characterized has been suggested. Thus, aim of this study was the comparison between two sperm selection method, HA binding and Zeta method, to select spermatozoa with normal morphology and intact chromatin
Materials and Methods: At present, sperm selection for ICSI only depends on morphology and motility, but these parameters may not be relevant the chromatin integrity. So sperm selection based on sperm functional characterized has been suggested. Thus, aim of this study was the comparison between two sperm selection method, HA binding and Zeta method, to select spermatozoa with normal morphology and intact chromatin
Results: Both HA binding assay and Zeta method are efficient to select sperm with normal morphology and lower protamine deficiency ($p<0.05$). But in term of DNA fragmentation Zeta method appear to be more efficient to select sperm with low DNA fragmentation ($p<0.05$).
Conclusion: The results of this study suggest that these sperm selection method can select spermatozoa with normal morphology and protamine deficiency and can use of selected sperm in ICSI. But Zeta method may be more efficient to select sperm with low DNA fragmentation. In patient with high DNA fragmentation, Zeta method can

be useful to select spermatozoa for ICSI

Keywords: Hyaluronic Acid (HA), Zeta Potential, Intra-Cytoplasmic Sperm Injection (ICSI), Morphology, Protamine Deficiency, DNA Fragmentation

O-21: Assessment of a New System for In-vitro Maturation of Human Cumulus-Enclosed Oocytes: Three-Dimensional Culture in the Presence of a Phosphodiesterase 3-Inhibitor

Dirckx K[1],Cabri P[1], Gerris J[1],Dhont M[1], De Sutter P[1], Vanhoutte L[1*], Dumortier F[1], Nogueira D[2]

1. Centre Reproductive Medicine,Ghent University Hospital, Ghent, Belgium
2. Laboratoire de Biologie, IFREARES, ToulouseFrance
Email: leen.vanhoutte@ugent.be

Objective: During conventional in vitro maturation (IVM) culture, oocytes undergo nuclear maturation but do not attain full cytoplasmic maturity. Previous studies showed that a prematuration culture (PMC) using a phosphodiesterase 3-inhibitor (PDE3-I) prevents spontaneous maturation and favours the synchrony between nuclear and cytoplasmic maturation. However, during the inhibition period, oocytes start to loose the connections with their surrounding cumulus cells. This may affect optimal maturation. In the present study, we intended to preserve oocyte-cumulus cells connections during the inhibition period by embedding cumulus-enclosed oocytes (CEOs) in an extracellular matrix (collagen). This so-called 'three-dimensional prematuration culture' (3D-PMC) was tested for its effectiveness to support IVM, fertilization and embryonic development.
Materials and Methods: Immature CEOs were retrieved from small antral follicles (diameters 5-10 mm), 34-36 h post-hCG from informed consenting patients who had undergone controlled ovarian stimulation for IVF/ICSI treatment. For the 3D-PMC, a neutralized collagen solution (Type I) was prepared. One microdroplet (5µl) of this gel was placed in the bottom of a 4-well culture dish and a single CEO was added to it. The gels containing the CEOs were placed in an incubator at 37°C to polymerize before adding 400µl of culture medium containing 1 µM PDE3-I (Cilostamide) to each well. The gels were cultured for 24 hrs at 37°C in a humified atmosphere of 5% CO2 in air. Afterwards, CEOs were removed from the gel, washed away from PDE3-I and underwent IVM for a maximum of 48h. Polar body (PB)-extruded oocytes were fertilized by ICSI and embryonic development was analyzed until day 3 post-ICSI. The results were compared to non-arrested, conventionally in vitro matured CEOs (in vitro control) and freshly collected, PB-extruded oocytes originating from small antral follicles from the same patients (in vivo control).
Results: A total of 196 CEOs were retrieved from 57 patients. Sixty percent (n = 118) of the oocytes were at the germinal vesicle (GV)-stage. These oocytes were distributed among the two IVM conditions. Twenty-nine percent (n=56) of the oocytes were at the PB-stage at retrieval and were considered in vivo controls. Oocytes embedded in collagen and prematured in PDE3-I-containing medium were efficiently arrested at the GV-stage (>90%). During the course of 3D-PMC, cumulus cells remained in close contact with each other and with the oocyte. After removal from collagen and inhibitor, oocytes were capable of resuming meiosis. At the end of IVM, 3D-PMC oocytes acquired higher maturation rates compared to the in vitro controls (81.6% versus 60.6%; $p<0.05$). Fertilization rates were significantly higher in the in vivo controls compared to the in vitro controls (76.8% versus 55.0%; $p<0.05$), but similar to the 3D-PMC (67.5%; $p>0.05$). On day 3 post-ICSI, a significantly higher proportion of good-quality embryos were obtained in the in vivo (55.8%) and 3D-PMC groups (55.6%) compared to the in vitro control group (27.3%; $p<0.05$).
Conclusion: Three-dimensional prematuration culture of human CEOs had a beneficial effect on oocyte developmental capacity, resulting in an increased yield of matured oocytes and an improved embryonic developmental quality.

Keywords: In Vitro Maturation, Oocyte Development, Phosphodiesterases, Three-Dimensional Culture

O-22: Molecular Construction of Tenecteplase Coding Sequence in Order to Express of its Product (as Thrombolytic Agent) in the Milk of a Transgenic Livestock

Dormiani K[1, 2], Khazaie Y[1*], Ghaedi K[1, 3], Nasr-Esfahani MH[1], Mir Mohammad Sadeghi H[2]

1. Transgenic Animal Research Group, Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
2. Pharmaceutical Biotechnology Department, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
Email: dormiani@yahoo.com

In this project, we have employed a rapid and efficient method to introduce three sets of mutation into defined positions in human tissue plasminogen activator (t-PA) cDNA sequence. A site-directed mutagenesis approach was done based on megaprimer PCR procedure to produce tenecteplase coding sequence. Tenecteplase is a variant with better pharmacokinetic properties and more selective thrombolytic activity. The final PCR-product was confirmed by agarose gel electrophoresis, restriction digestion and sequencing. At the next step, a fragment containing of this sequence was cloned into pTZ57R/T by T/A cloning method and propagated in One Shot® TOP10 chemically competent E. coli and the positive

clones were selected by blue/white screening method. After isolation and purification of recombinant plasmid and its digestion by suitable restriction enzymes, tenecteplase coding sequence was purified and subcloned into an appropriate vector. This vector contains a tissue specific promoter for producing of recombinant proteins into the milk of a determined transgenic livestock. The recombinant vector was propagated in One Shot® TOP10 chemically competent E. coli. Finally, recombinant plasmid was isolated and verified by restriction digestion and sequence analysis to confirm that the insert has been cloned in the proper orientation and contained the appropriate features required for expression into the milk.

Keywords: Tissue Plasminogen Activator, Tenecteplase, Thrombolytic, Megaprimer, Site Directed Mutagenesis, Transgenic

O-23: Lipofectamine Does Not Enhance Bovine Sperm Transfection Efficiency

Eghbalsaied Sh[1*], Ghaedi K[2, 3], Nasr-esfahani MH[2], Mozafari NA[4]

1. Animal Science Department, Agricultural College, Khorasgan branch, Islamic Azad University, Isfahan, Iran
2. Clinical and Experimental Embryology and Stem Cell Research, Cell Sciences Research Center, Royan Institite, ACECR, Tehran, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
4. Microbiology Department, School of medicine, Iran University of Medical Sciences, Tehran, Iran
Email: kamranghaeedi@yahoo.com

Objective: (i) if using chemical reagents such as DMSO and Lipofectamine™2000 increase bovine sperm transfection, (ii) if motile spermatozoa has a greater transfection rate than immotile ones, and (iii) if increasing sperm-DNA incubation time enhance sperm transfection rate.
Materials and Methods: Motile bull sperms were allowed to swim up at 38˚C for 90 min. The rhodamine labeled pGeneGrip-Lac-Z vector (Genelantic, USA) was used for direct detection of transfected cells under epiflourescence microscope. One and three microliters of Lipofectamine2000 were added separately into two tubes containing 20µl of SP-TALP medium (BSA and antibiotic free) and stored at room temperature for 30 min. One microgram of the vector was added to liposomes and stored at room temperature for a further 90 min to perform 1:1 and 1:3 (DNA:Lipofectamine) ratios. one µg of naked DNA was added into SP-TALP medium as control group and supplemented with 1% and 3% Dimethyl sulfoxide(DMSO) as DMSO groups. for all groups, 1×106 fully capacitated spermatozoa was added to each tube and the total volume adjusted to 30 µl with SP-TALP medium (containing 6% BSA) and incubated at 38°C for 60 min. Sperm-DNA mixtures were divided into two groups, treated with or without DNaseI (5 UI) for 30 min and used for fluorescence detection. In another experiment, motile sperms were separated through Pure-Sperm gradient after sperm-DNA incubation. The effect of sperm-DNA incubation time on transfection rate was evaluated by detecting transfected sperms at 15, 30, 60, 120, 150, 180, 210 and 240 min of incubation. Data was analyzed using general linear model (GLM) procedure and and Pair wised t-test by SAS package.
Results: sperm transfection rate in control, DMSO1%, DMSO3%, Lipofectamine 1:1, and Lipofectamine 1:3 were 24, 31, 37, 20 and 0 percent, respectively. DNaseI treatment removed DNAs bounded at the acrosome and partial parts of sperms. Immotile sperms have significantly greater transfection rate than motiles in all groups. Increased both DMSO and Lipofectamine ratios could increase sperm-DNA bounding and uptake. Transfection rate has a steady status from 15 to 90 min. however, prolonged incubation time (>90 min) led to decrease in transfection rate and fixed at 15% after 4 h incubation.
Conclusion: The maximum of transfection rate for bovine sperms obtained with DMSO3%. In contrast Lipofectamine2000 could not improve sperm transfection rate in our experiment conditions. Incubation of sperm and DNA for 15 min is sufficient to have acceptable transfection efficiency.

Keywords: Transfection, Lipofectamine, DMSO, Sperm and Bovine

O-24: IUI and Lab-which Laboratory Gives a Better Outcome?

Mohanna SAM

Mazandaran University,Hevdahe Shahrivar Hospital, Amol, Iran
Email: seyabb_mirmo@yahoo.com

Objective: IUI is frequently used as a first line strategy in the treatment of infertile couples because of its relatively low cost and simplicity. Unfortunately, this cooperation without considering enough standard laboratory equipments, materials, and methods leads to unexpected and risky results which are not desirable. Because of preventing undesirable results it should be done in the hands of expert and knowledgeable persons. As it can be performed efficiently in the office and does not require sophisticated equipment (but standard) for diseases such as endometriosis, male infertility and anovulation.
Predictive sperm parameters and threshold values with respect to semen characteristics for successful intrauterine insemination have been controversial, although the world health organization's reference values for semen analysis are often use to assess sperm quality. More often pregnancies which are not successful with IUI have been achieved in subfertile couples with sperm parameters below reference values listed in the W.H.O. manual. One of the most important parameters in a successful IUI is the precise knowledge of cooperative lab. about the full

structure and morphology of sperm. The sample should be processed under standard conditions to avoid using all the negative parameters and shocks, in order to gaining a desirable outcome. Sperm processing techniques for IUI vary from laboratory to laboratory and even from patient to patient. IUI has been used with variable success for the treatment of numerous indications in the infertile couples. Accordingly several semen parameters have been essential for successful IUI as mentioned below: 1. The semen should have the minimal reference values of W.H.O. 2. T.S.M. In the first and processed sample has important prospective. 3. Material and method should be standard and done in a standard lab. 4. Speed and forward progression percentage of sperms. 5. Making the right decision for choosing ether of swim up or gradient method for sperm processing. 6. Knowing the probable defects on a fetus due to laboratory mistakes.
Materials and Methods: Prospective study
Results: there should be a permanent observation and contribution between the lab responsible for IUI and responsible performed IUI clinics
Conclusion: without consideration of obligate steps for IUI, there might be the risk of genetic defects in the children who were born after such IUI performances.

Keywords: IUI, Laboratory

O-25: The Effects of Vitrification on Mouse Blastocyst DNA Fragmention

Rajaei F[1*], Soleimani Rad J[2], Ghaffari Novin M[3], Niknafs B[2], Naseri S[1], Rezazadeh MR[1]

1. Qazvin Infertility Centre, Kosar Hospital, Qazvin University of Medical Sciences, Qazvin, Iran
2. Department of Anatomy & Drug Applied Centre, Tabriz University of Medical Sciences, Tabriz, Iran
3. Cellular & Molecular Biology, Research Centre, Shahid Beheshti University M.C., Tehran, Iran
Email: farzadraj@yahoo.co.uk

In recent years there have been significant advances in vitrification for storage of embryos but there are not a reliable protocol in vitrification that result in high survival rate of embryos because the mechanisms of embryo injury has not still been cleared following the vitrification. The aim of the present study was to assay the effect of vitrification on DNA fragmentation in mouse flushed blastocysts. For this porpuse, 95 mouse blastocysts were obtained by flushing and were randomly divided into control and experimental groups. 52 Control group blastocysts were cultured in M16 media for 2 hours and then the apoptotic index were obtained after staining by TUNEL technique. 43 experimental group blastocysts were vitrified in EFS40 solution and kept in LN2 for one month. After thawing and culture in M16 for 2 hours, the apoptotic index were obtained by TUNEL staining. The DNA fragmentation indexes in two groups were analyzed by statistical methods.
The results showed that the DNA fragmentation index in vitrified blastocysts were significantly higher than control group blastocysts (9.2 vs 11.9, $p<0.004$). It can be concluded that the vitrification can increase DNA fragmentation cell death in mouse blastocysts.

Keywords: Vitrification, DNA Fragmentation, Mouse Blastocysts, TUNEL

O-26: Differential Expression of TGFBR3 (Betaglycan) in Mouse Ovary and Testis During Gonadogenesis

Sarraj MA[1*], Umbers A[1], Findlay JK[1], Stenvers KL[1],Chua HK[2]

1. Prince Henry's Institute,Female Reproductive Biology, Clayton, Victoria, Australia
2. Prince Henry's Institute, Female Reproductive Biology, Clayton, Victoria
Email: mai.sarraj@princehenry.org

Objective: TGFBR3 is an accessory receptor that binds to and modulates the activities of both TGFbeta and inhibin, two members of the TGFbeta superfamily of growth factors that regulate many aspects of reproductive biology. Tgfbr3 is known to be expressed in adult testis and ovary, but little is known about this receptor during gonadogenesis. Herein, we describe Tgfbr3 expression in the male and female fetal and neonatal murine gonad.
Materials and Methods: We have employed real time analysis and immunofluorescence to determine the expression pattern of TGFBR3 (betaglycan) in the mouse ovary and testis during gonadogenesis.
Results: Real-time PCR analysis revealed that Tgfbr3 mRNA was expressed at higher levels in the developing testis compared to ovary. TGFBR3 was expressed within the fetal testis interstitium, predominantly by Leydig cells, but expression shifted inside the seminiferous cords at birth. In contrast, TGFBR3 was detected in both the somatic and germ cell lineages in the fetal and neonatal ovary.
Conclusion: TGFBR3 differential expression pattern suggests divergent roles for this TGFBR3 in developing testis and ovary.

Keywords: Betaglycan, TGFbeta Type III Receptor, Inhibin, Leydig Cell, Gonadogenesis

O-27: An Optimized Method to Obtain Mature Oocytes After Human Ovarian Xenotransplantation into the Back Muscle of SCID Mice

Soleimani R[1*], De Sutter P[1], Heytens E[1], Dhont M[1], Gerris J[1], Cuvelier C[2]

1. Center for Rep, Med, Uro-Gynecology, Gent, Belgium
2. Gent University, Pathology, Gent, Belgium
Email: rezasoleimani@yahoo.com

Objective: The only option for fertility preservation in young female cancer patients is cryopreservation of ovarian tissue(OT), containing primordial follicles, followed by grafting of frozen-thawed OT. Recently we introduced the back muscle (B) as a favorable site for mouse OT allografting and human OT xenografting. The aim of the present study was to optimize the method of xenotransplantation of human OT to SCID mice. Xenograft survival, follicular atresia due to apoptosis, vascularization and follicular development were compared between the B-site versus under the kidney capsule (K).
Materials and Methods: Thin Cryopreserved OT-strips from a 22-year old female-to-male transsexual person were thawed, washed and cut to small pieces (~1mm×1mm) (Thickness 432±145µm; mean±SD). Follicular content of the strips was evaluated using a glass-bottom dish under a stereo microscope. OT-strips with minimum five follicles were selected randomly and transplanted to B or K-sites in SCID mice. 1) Five OT fragments were transplanted to each site (B&K) in eight mice to study apoptosis during the first eight days of transplantation. Grafts were collected daily and processed for immunohistochemical staining with anti-active-Caspase-3-(AC-3). 2) In 28 mice, follicular development in B and K xenografts was evaluated after 3, 5 and 7 months. Animals were injected every second day with 1 IU FSH starting one week after grafting. Graft samples were processed for hematoxylin-eosin (H&E), Human α-smooth muscle actin (α-SMA) and mouse-CD31, human-CD34, PCNA and AC-3 immunohistochemistry. From the 5th month, follicular development in both B&K-sites in all animals were evaluated by magnetic resonance imaging (MRI) every two weeks. Animals with follicles >6mm were injected every second day with 5 IU FSH for two weeks followed by one dose of 10 IU HCG. 36h later animals were autopsied and grafts were collected for histology. 3) In a complementary study, two follicles from B-grafts (>6mm) were punctured.
Results: 1) In general higher numbers of AC-3 positive follicles were seen in K-Grafts from day 3-8 and at 3-7 months after grafting. 2) Higher numbers of MII oocytes were obtained from B & K-grafts in the group that was stimulated after MRI evaluation. Higher PCNA positive follicles were observed in B-grafts. There was a correlation between number of blood vessels and size of follicles in the grafts. Vascularization was more prominent in B-grafts (9.1±1.7 versus 4.2±0.74, $p<0.01$). Higher numbers of α-SMA positive vessels were seen (3.96±0.33 versus 1.52±0.25, $p<0.001$). Blood vessels positive for α-SMA, human-CD31 and mouse-CD34 were evidence of anastomosis of existing human vessels and invading murine vessels in OTs. 3) One MII-oocyte and one GV-oocyte were obtained. The GV-oocyte became MII after in vitro maturation (IVM).
Conclusion: Conclusion: Human and murine blood vessels are present after grafting in a larger extent in B. Lower numbers of AC-3 positive follicles in B-grafts confirmed better prevention of ischemia due to advanced support of murine and human blood vessels. B allows larger follicle size development than K. As far as we know this is the first report of an MII and IVM-MII oocyte obtained from a human antral follicle after xenografting to the B-site.

Keywords: Human Ovarian, Tissue Cryopreservation, Tissue Transplantation

O-28: Peroxisomal Protein cDNA Cloning: Toward Understanding of its Molecular Aspects

Tanhaaii S[1*], Ghaedi K[1, 2], Karbalaii Kh[1], Razavi Sh[3], Nasr-Esfahani MH[1], Rabiee F[1], Baharvand H[1]

1. Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
2. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
3. Anatomical Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
Email: kamranghaedi@yahoo.com

Peroxisomes are tiny organelles distributed in the almost all eukaryotes from yeast to human. One of the Peroxisomal functions is plasmalogen biosynthesis which are major components in nerve cell membranes. There is a close relation with peroxisome biogenesis and neuronal development and migration. Peroxisomal biogenesis disorders (PBDs) are the best examples that show disturbance in peroxisomal function leads to severe neurological dysfunctions in newborns who are suffering from these disorders. Identification of peroxisomal matrix proteins and their imports have deepened our understanding of the molecular mechanisms of protein kinesis in peroxisomes. In order to study the molecular mechanisms of peroxisome biogenesis and regulation of its function, through neuronal differentiation and development, we have recently cloned murine cDNA of a novel peroxisomal protein termed PEP. PEP cDNA was inserted in to the pEGFP-C1 downstream the EGFP cDNA under regulation of CMV promoter. Mouse PEP protein contains tri-peptides, SKI, resembling peroxisomal targeting signal type (PTS1) at its C-terminus. In order to assess the functionality of that tripeptides and intracellular localization of EGFP-PEP, CHO-K1 and P19 cells were transfected with the constructed plasmid. Data showed that PEP was localized in peroxisomes. Bio-informatics analysis revealed the presence of two hydrophobic domains in this protein which their functions remain to be elicited. Interestingly there is a Fibronectin type III domain in this protein which is a potential site for protein-protein interaction.

Keywords: Fibronectin, PEP cDNA, Peroxisome, PTS1 Signal

O-29: Nuclear and Cytoplasmic Maturation of In Vitro Matured Human Oocytes After Temporary Nuclear Arrest by Phosphodiesterase 3-Inhibitor

Vanhoutte L[1*], De Sutter P[1], Gerris J[1], Dhont M[1], Nogueira D[2], Van der Elst J[3]

1. Centre Reproductive Medicine, Ghent University Hospital, Ghent, Belgium
2. Lab de Biologie, IFREARES, ToulouseFrance
3. Centre Reproductive Medicine, Brussels University, Brussels, Belgium
Email: Leen.Vanhoutte@ugent.be

Objective: The use of hormones for controlled ovarian stimulation results in follicular heterogeneity, with oocytes at diverse stages of nuclear and cytoplasmic development. This study evaluated the impact of temporary nuclear arrest by a specific phosphodiesterase 3-inhibitor (PDE3-I), Cilostamide, on nuclear and cytoplasmic maturation of cumulus-free germinal vesicle (GV) human oocytes from controlled ovarian stimulated cycles.
Materials amd Methods: GV oocytes were culture in: (1) medium without the inhibitor (control); (2) medium supplemented with 1 μM Cilostamide and (3) medium supplemented with 10 μM Cilostamide. Oocytes in groups (2) en (3) were exposed to Cilostamide for 24 h. The PDE3-I was subsequently removed by transfer of oocytes to fresh in vitro maturation (IVM) medium and the reversibility of GV arrest was assessed during IVM culture for maximum 48 h.
Results: Cilostamide (1 and 10 μM) could maintain >80% of the oocytes at the GV stage, without affecting subsequent maturation to metaphase II (MII). Oocytes exposed to 1 μM Cilostamide were more likely to have normal bipolar spindles with aligned chromosomes than control oocytes ($p<0.05$). When GV chromatin configurations before and after arrest were compared, a significantly higher proportion of oocytes had acquired a nucleolus completely surrounded by a rim of highly condensed chromatin ($p<0.05$).
Conclusion: Temporary nuclear arrest of human GV oocytes with PDE3-I proved to be beneficial for obtaining normal spindle and chromosome configurations after IVM. It resulted also in synchronization within the population of GV oocytes.

Keywords: In Vitro Maturation, Meiosis, Oocyte Maturation, Phosphodiesterases, Spindle

Epidemiology and Ethics

O-30: Ethics and Commercial Surrogate Motherhood

Dekovic S

Clinical Center of University, Gynecology/Obstetric Clinic, Sarajevo, Bosnia and Herzeg
Email: sanjindekovic@yahoo.com

Objective: Surrogate motherhood represents one of the most controversial forms of commercialization in reproduction. Since it was first applied twenty-three years ago in Great Britain, it has been a subject of numerous ethical, medical and legal discussions, and its outcomes have often had epilogues in long-lasting court proceedings.
Materials and Methods: Consideration of surrogate motherhood in light of numerous ethical dilemmas that this form of reproduction brings.
Results: In the case of commercial surrogate motherhood there are analysts who are in favor, and those who are explicitly against this method of producing offspring. Thus the advocates of surrogate motherhood believe it is acceptable in case it does not cause harm to any other being, and also that every female person has the right to have children, even through surrogate motherhood, if that will make her happy, and therefore surrogate motherhood should be allowed in the whole world. Opposed to this are the viewpoints that surrogate motherhood is not ethical, as it represents parenthood for profit, exploits women of weaker material status, reduces women's honor. From the ethical point of view, there are additional imposing questions, such as: what should be done if a surrogate mother decides to keep the child? What if she wants to visit the child? Is giving a baby over after delivery actually a classical sale? Is renting of surrogate mothers moral and under which conditions, and what kind of women could be surrogate mothers? If we assume that surrogate motherhood is actually legally permitted, a question is raised whether that would result in certain women simply deciding to, out of pure convenience, produce all their children in a way that their child is carried by another person. Considering that in most cases it is the women of lower economic class who apply to be surrogate mothers, such contracts could perhaps lead to another division between the rich and the poor. A separate issue is the morality of a surrogate mother and her approach to pregnancy in relation to her previous way of life. On the other hand, the necessary ethical approach of contractors – customers of a surrogate mother is not negligible in cases when she gives birth to a disabled or malformed child, or becomes ill during pregnancy, and suffers death in pregnancy or at labour.
Conclusion: A desire for motherhood is one of fundamental aspirations that accompany the mankind through centuries. One of the ways to realize it in the last decades has also been the commercial surrogate motherhood. Still, it is prohibited in a large number of countries as an unethical method of trade in human life, together with the opinion that it represents exploitation of women and their organs. However, the justification of such a solution needs to be considered in couples that due to objective reasons have surrogate motherhood as the only option. Considering a series of not only ethical, but also medical, legal and social controversies that accompany commercial surrogate motherhood, it should be strictly limited by a reaction of the criminal law. That would prevent manipulations, and a possibility for such contracts to result in long-lasting court proceedings that will impair the quality of life of not only the contractors and the surro-

gate mother, but also of the child born this way.

Keywords: Surrogate Mother, Ethics

O-31: Public Opinion Regarding Oocyte Donation in Iran

Fereydouni B[1*], Fereydouni Sh[2], Solimani H[3]

1. Biology Department, College of Sciences, Shiraz University, Shiraz, Iran
2. Education Psychology Department, College of Humanity, Gachsaran University, Gachsaran, Iran
3. Education Psychology Department, College of Humanity, Shiraz University, Shiraz, Iran
E mail:b.fereidooni@gmail.com

Objective: There are a number of different assisted reproduction source, embryo source or even as a carrier of pregnancy. Third party assisted reproduction treatment has been one of the more contentious issues surrounding assisted reproduction,
eliciting active debate within many societies with regard to its moral, ethical and religious implications. Oocyte donation may be a treatment option for women
having had cancer treatment, women with premature ovarian failure, peri- and post-menopausal women, women who are known carriers of a gene for serious X-linked disorders and autosomal conditions, women with poor oocyte and/or embryo
quality or multiple failures during prior attempts to conceive by means of assisted reproduction treatment.
Materials and Methods: This descriptive study was carried out in 2007-2008 in five regions (south, north, west, east and center of the city of Shiraz). Two hundred participants (>18 years old) were chosen by cluster Sampling method.
Results: A total of 200 respondents constituted the study group, 100 (50%) women and 100 (50%) men. The mean age was 29.40 4.67.62.5% were married, 36.5% were single.75% women and 71%men did not have any knowledge about oocyte donation.57% had university graduations, 27% had diploma from high school and 15.5% with out diploma.25% of women and 57%of men under no circumstances accepted this kind of treatment to do.4% women and 2% men accepted if they had inherited problems in family. 2% women and 2% men accepted if they had disabled child. 64% women and 36% men accepted due to women age.
Conclusion: The present results indicate that, in general, Iranian people support oocyte donation in some cases as an alternative way of starting a family. Clinics in many countries have difficulties in recruiting altruistically motivated fertile women who would like to donate, and use different strategies for recruitment.

O-32: Profiling Sexual, Reproductive and Mental Health of Islam Adolescent Girls

Jaiboon S[1*], Hussain M[2]

1. Total Recons of Environandec (NOG), Women Health & Reproductive Health, Ananthapur, India
2. Swarnandrabharathi College, Biochemistry, Gudur, Inda
Email: jaiboon@rediffmail.com

Objectives: The objectives of the study are aimed to make significant forum through convention of adolescent Islam girls understand misconceptions, patterns and consequences of risky sexual behavior, providing sexuality education. Focus character based sex education, awareness to consequences of pre marital sex to make understand risks of STI, STD and HIV through highlighting abstinence education. Building character, moral, ethical, physical, social perceptions and control emotion among target population.
Materials and Methods: 400 islam adolescent students were selected as target group. Since, the reproductive age starts with adolescent stage the investigator selected 13-18 years age group in his conventional study. In understanding the perspectives of young girls in the cultural context, major issues associated with transition from child hood to adult hood period, physical, physiological and psychological changes and its misconceptions, patterns and consequences are identified. The identified risks, projected with perfect protocol on pre marital sex, STI, STD and HIV consequences through abstinence education and building character, moral, ethical, social and physical parameters through emotion control. The study protocols are measured with suitable research tools and statistical techniques.
Results: The conventional study on sexuality education, premarital sex consequences and risks of STI, STD and HIV revealed highly positive response among Islam young girl's misconceptions and patterns related to transmission period from childhood to adulthood. The selected psychological parameters character, moral and ethical values are developed and emotion control witnessed due to study interventions. Progress in physical and social health perceptive among the target population.
Conclusions: From the study findings it is concluded that systematic and scientific conventional research approach will give very positive results among adolescent girls sexual reproductive and mental health.

Keywords: Indentification of Adolescent Age Misconceptions, Patterns and Conseqences Charector Based Sex Education Bulding Charector, Moral and Ethical Values Abstinence Education Adulthood Transition Changes

O-33: Significance of Human Chorionic Gonadotropin in the Growth and Maturation of Preantral Follicles and Enclosed Oocytes During Optimized In Vitro Cultures: A Randomized Study

Javed AJ[1*], Rezaei-Zarchi S[2], Javeed Ghani M[3], Kalantar SM[4*]

1. University of Agriculture, Biochemistry Department, Faisalabad, Pakistan
2. Payam-e-Noor University, Biology Department, Yazd, Iran
3. Government College University, Bioinformatics Department, Faisalabad, Pakistan
4. Research Center of Infertility, Biotechnology Department, Yazd, Iran
Email: libraforever_2006@yahoo.com

Objective: The study was carried out to evaluate the effect of hCG, in the presence and absence of FSH, on the timing and regulation of in vitro ovulation in Syrian mice preantral follicles.
Materials and Methods: Preantral follicles, isolated from the ovaries of 6 week-old mice, were cultured in TCM-199 medium. The effect of 10-200 mIU/ml FSH and 1.5 IU/ml hCG, the follicles were incubated for 6 days at 37 °C, 92% humidity and 5% CO2 in air.
Results: FSH concentration of 100 mIU/ml showed increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates ($p<0.0001$). A significantly higher number of follicles showed mucified cumulus cells, attached to the oocytes when ovulation started within 16-24 hours post hCG (97 and 80%, respectively; $p<0.0001$). Successful ovulation failed to occur when the follicles were allowed to ovulate without hCG administration or more than 24 hours post hCG administration. While, in the medium containing FSH and 1.5 IU hCG, the ovulation percentage reached a maximum of 97% as compared to that seen for FSH-containing medium only (81%) or in control experiment (10%). So, the effect of FSH + hCG was highly significant over the control medium ($p<0.0001$).
Conclusion: Recombinant hCG and FSH are effective in promoting oocyte maturation in a clinical IVM program when administered in combination.

Keywords: Follicle Stimulating Hormone, HCG, Preantral Follicles, Oocyte Maturation, GVBD

O-34: Profiling Health Reproductive Health Spirtual Health and STI/STD Programmes for Islam Women Beedi Workers

Najeebullam M

NBKR College, Health and Physical Education, Vidyanagar, India
Email: m_najeebullah@rediffmail.com

Objectives: The objectives of the programme are aimed to make Visible change and update Islam Women Beedi Workers Physical, Mental, Spiritual and social health through exercise and Meditation. Mapping and implementing reproductive health best practices, family planning, promote health service programmes to bring down morbidity rate and STI/STD. Develop Policy action tools for gender equity and reproductive rights, reduce female child abortions and bring perceptible changes and responsibilities among their male spouses (husbands).
Materials and Methods: 500 Islam women beedi workers aged 20 – 40 years participated in this programme, implemented through suitable protocol with the help of Doctors, Paramedics, Nutritionists and physical exercise and Meditation experts. To promote selected health parameters Physical, Mental, Spiritual, Social and reproductive health, STI/STD management family planning and nutrition programmes were implemented among the target population. The pre test and post test interventions are measured to find out the progress on the selected parameters with suitable statistical applications.
Results: The implemented programme protocol clearly states significant results in health, reproductive health, mental, spiritual and social health among the Islam women beedi workers. Progress in family planning programmes, female child abortions and morbidity rate was found. Perceptible changes among the male spouses behavior also found through policy action tools implementation. Awareness of nutrition and hygiene activities and control to STI/STD found to be increased among the target group.
Conclusions: From the study findings it was concluded that systematic and scientific health service programmes will give significant results and bring perceptible changes in family planning nutrition, hygiene and STI/STD among low socio-economic female groups as well as their male spouses.

Keywords: Spritual Health, Female, Child, Abortions, Familyplanning Programmes, Males, Spouses Behaviour, Meditation

O-35: Debate in Embryo Donation: Embryo Donation or Both Gamete Donation?

Samani R[1*], Rezania Moalem MR[1], Merghati ST[1, 2], Alizadeh L[1]

1. Cell Sciences Research Center, Ethical Committee, Royan Institite, ACECR, Tehran, Iran
2. SAMT organization, Tehran, Iran
Email: samani@royaninstitute.org

So far, more than 2 million babies have been born worldwide through assisted reproductive technologies (ART). For many couples there is no treatment except joining a third party to the family. Recently, embryo donation law has been approved by Iran's parliament and now it is legal in Iran. But there is a misunderstanding in the source of embryos: from surplus frozen embryos of infertile couples and sperm and egg donation from fertile married couples followed by donation of the resulting embryos. Here in this paper we discussed ethical, religious and legal aspects of these two procedures and present the advantages and disadvantages of them. Meanwhile, the new term "both gamete donation" was defined for the misplaced program instead of "embryo donation". In conclusion we can say: 1) Iranian law means only embryo donation and covers only surplus embryos from

other infertile couples and not both gamete donation. 2) As gamete donation is practiced in Iran upon decrees of clergy leaders, there is nothing against both gamete donation. 3) There are so many ethical, legal and religious questions about "both gamete donation" to be answered. 4) Ethical and religious questions are very fewer about "embryo donation" comparing to "both gamete donation" program, and 5) Embro sharing is a good way for donation of fresh embryos.

Keywords: Egg Donation, Gamet Donation, ART

O-36: Anxiety During Pregnancy in Primgravidas and Women with History of Fetal or Neonatal Death

Taavoni S[1*], Aahadie M[2], Ghanjie T[3], Hoseinie F[4]

1. IUMS, Midwifery Group, Tehran, Iran
2. Mashad UMS, Health Center, Mashad, Iran
3. IUMS, Nursing Group, Tehran, Iran
4. IUMS, Statistic Group, Tehran, Iran
Email: staavoni14@yahoo.vom

Objective: Although pregnancy is lovely events in the developmental processes of a woman's life, history of previous fetal or neonatal death can have a negative effect on the adaptation of a woman for her new pregnancy and produce irretrievable effects. AIM: To assess and comprise anxiety in primigravidas and pregnant women with the history of previous fetal or neonatal death.
Materials and Methods: A two group comparative design and nonrandomized sampling method (sequential) was used. We collected 120 Iranian pregnant women with a basic education, who were in 3 rd trimester of their planed current pregnancy (20–40 years old age). 40 of samples had previous history of fetal or neonatal death (without any live child) and 80 of them were primgravidas. The tools, which was used for this study had two main parts: personal characteristic, and pregnancy outcome questionnaire (POQ of Theut et. al. 1988
Results: This study only found significant difference in 8 of 15 statements of POQ between tow groups. The average of anxiety during pregnancy in the pregnant women with previous fetal or neonatal death was more than second group. The independent t-test also showed a significant difference between tow groups (p=0/000).
Conclusion: Due to the increase of anxiety in pregnant women with previous history of fetal or neonatal death, it sounds it is necessary to plan supportive, educational and counseling program for the mentioned high risk group of women. We suggest to continue the same research during the first and second trimester of pregnancy and postpartum in the clients whom will be visited in the other clinics.

Keywords: Anxiety, Pregnancy, Fetal Death, Neonatal Death

O-37: The Effect of Counseling on Quality of Marital Relationship in Infertile Couple who Are Visited of Vali-e-Asr Reproductive Health Research Center in Tehran

Vijeh M[1*], Pakgohar M[2], Ramezanzadeh F[3], Babaee GR[4], Abedininia N[5]

1. Faculty of Midwifery, Azad Mahabad University, Mahabad, West Azarbaijan, Iran
2. Faculty of Midwifery, Tehran University of Medical Sciences, Tehran, Iran
3. Obstetrics and Gynecology Department, Vali-e-Asr Reproductive Health Research Center ,Tehran University of Medical Sciences,Tehran, Iran
4. faculty of Tarbiat Modarres University, Tehran, Iran
5. Clinical Psychologist, Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciencs, Tehran, Iran
Email: m_vijeh@yahoo.com

Objective: Infertility is a major factor that leads to sexual and marital dissatisfaction. In many couples , the infertility crisis can be seen as a cumulative trauma, which indicates that these couples have a marked need for infertility counseling. The purpose of this study was to determine the effect of counseling on infertile couple's sexual and marital satisfaction that referred to the fertility center in Tehran in 2007.
Materials and Methods: It was an interventional study with use of control group.The 100 infertile couples who had eligible criteria , were recruited in this study by convenience sampling method and then randomly allocated in to two groups (50 couples in counseling and 50 couples in control groups). Study was designed two phases pretest before intervention and was followed up 3 months later. Counseling group participated during 3 hours through 3 sessions every week. Data was collected by 3 questionnaire include: demographic, marital satisfaction questionnaire and sexual satisfaction questionnaire .As a results, higher scores of sexual and marital satisfaction questionnaire revealed lower satisfaction. Data were analyzed with SPSS software and $\chi 2$, Mann Whitney and willcoxon test ($p<0.05$).
Results: The average of sexual satisfaction in the women of counselling group was mean±sd =36.00±8.37 and in the control group was mean±sd =40.04±7.69. There was a significant difference between two group 3 months after intervention (U=741.500, p=0.019).
The research findings revealed that the average of sexual satisfaction in the men of the counseling group was mean±sd =33.37±7.09 and in the control group was mean±sd =36.63±6.52 and Man-Whitney test (U=746.500, p=0.02) indicated that there is a significant difference between two groups 3 months after intervention. The average of marital satisfaction in the women of the counseling group was mean±sd =50.23±11.8 and in the control group was mean±sd =54.97±12.64. We have obtained a considerable difference between two groups 3 months after intervention by man-Whitney test

(U=776.000, p=0.036).
The average of marital satisfaction in the men of the counseling group was mean±sd =45/9±10.1 and in the control group was mean±sd =50.08±11.43. There was a significant difference between two group 3 months after intervention (U=758.500 , p=0.022).
Conclusion: The results of this study supported that infertility counseling increased the sexual and marital satisfaction of infertile couples.

Keywords: Counseling, Sexual Satisfaction, Marital Satisfaction, Infertility

Female Infertility

O-38: Pentoxifylline Therapy after Laparoscopic Surgery for Different Stages of Endometriosis: A Prospective, Double Blind, Randomized, Placebo-Controlled Study

Alborzi S

Obstetrics and Gynecology Department, Shiraz University of Medical Sciences, Shiraz, Iran
Email: alborzis@sums.ac.ir

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).

Keywords: Pentoxifylline, Endometriosis, Endometrioma, Cumulative Pregnancy Rate, Infertility, Immunomodulation

O-39: Laparoscopic Metroplasty in Bicornuate and Didelphic Uterus

Alborzi S

Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran
Email: alborzis@sums.ac.ir

Objective: To determine feasibility of laparoscopic metroplasty in treatment of bicornuate and didelphic uterus.
Design: Case report.
Setting: University and private hospitals.
Patients: Four women with a diagnosis of double uterine cavity (2 bicornuate and 2 didelphic uteri) with a history of two recurrent spontaneous abortions less than five months of pregnancy.
Intervention: Laparoscopic metroplasty with diagnostic hysteroscopy was performed for the unification of the uterus. Second look laparoscopy and hysteroscopy was performed about three months later.
Main Outcome Measures: Evaluation of the uterine compliance to high intrauterine pressure, presence of adhesions in pelvic and uterine cavity.
Results: In all four patients, laparoscopic metroplasty results in a unified uterus with a good cavity and tolerance to high intrauterine pressure. Minimal pelvic adhesions were noted in the two patients at the second surgery.
Conclusions: This new technique of laparoscopic metroplasty is an acceptable
alternative for abdominal meroplasty, with minimal adhesion formation.

Keywords: Adhesion Formation, Bicornuate Uterus, Didelphic Uterus, Laparoscopy, Metroplasty

O-40: TLR9 in Ciliated Cells of Human Fallopian Tube

Ghasemi N[1], Aflatoonian A[2], Aflatoonian R[3]

1. Yazd IVF Centre, Genetic Department, Yazd, Iran
2. Yazd IVF Centre, OB and Gy, Yazd, Iran
3. University of Sheffield, OB and Gy, Sheffield, UK
Email: n479@yahoo.com

Objective: The innate immune system evolved to detect

and respond to infection. Several families of receptors detect highly conserved pathogen-expressed molecules that are not expressed by or accessible in host cells. One of the best understood of these families is the TLR family of molecules, of which ten have been identified in humans. Each TLR binds to one or more distinct pathogen-expressed molecules and can function as an "alarm signal" for the immune system, initiating appropriate host immune defenses. TLR9 seems to evolve in detection of the unmethylated CpG dinucleotides that are relatively common in bacterial and viral genomic DNAs but are uncommon in vertebrate genomes and, if present, are highly methylated.
Materials and Methods: Normal fallopian tubes were detected by H&E staining, which blocks obtained from patients undergoing abdominal hysterectomy for benign gynecological conditions. Using immunohistochemistry techniques, distribution of TLR9 was studied in blocks of the fallopian tubes.
Results: Result showed TLR9 was present in epithelium especially in ciliated cell of fallopian tube intensely. The intensity of staining was not equal in cases.
Conclusion: Regulation of TLR9 expression and signaling may play an important role in the control of responses to luminal microorganisms. TLR9 stimulation innate immunity, which could make good defense against infection or tumor.

Keywords: Cliliated Cell, Fallopian Tube, TLR9, Innate Immunity

O-41: Practice of Female Population Towards Breast Cancer: An Experience at a Tertiary Care Hospital in Rawalpindi

Gilani S[1*], Mazhar T[1], Mir Sarah T[1], Sana a[1], Tariq Sofia, ST[1], Malik Asif, AZ[2]

1. Rawalpindi Medical College, Student, Rawalpindi, Pakistan
2. Rawalpindi Medical College, Surgery, Rawalpindi, Pakistan
Email: sigilani@hotmail.com

Objectives: The study was aimed to assess the awareness about breast self examination (BSE) and coverage of breast screening investigations in a developing country.
Materials and Methods: A cross sectional study was carried out at Holy Family Hospital, a tertiary care health facility at Rawalpindi interviewing 637 randomly selected adult females among the patients and their accompanying attendants, excluding patients with breast complaints. Data was collected using a structured questionnaire and analyzed using SPSS 13.
Results: The average age of participants was 32 years, majority of them (90%) were married, house wives (89%) with some education and urban dwellers with middle socioeconomic status. Only 27% (n=173) of the females knew how to do a BSE and practiced it. The main reason for not doing a BSE was lack of knowledge about it (54%, n=343). 21% (n=136) thought that they don't need to do a BSE and 15% (n=97) said that they don't have a breast complaint so it was useless to do n examination. Only 13% (n=80) have had a breast clinical examination by a doctor which was when they had a breast problem or in pregnancy. An even less number (5%, n=32) had a breast investigation (mammography n=11, FNAC n=5, ultasonography n=1). Though there was unanimous agreement (82%, n=524) among females that early detection of breast cancer can lead to a favorable outcome.
Conclusion: Despite wide agreement over the need for early detection of breast cancer, the knowledge and practice of BSE is very low and the coverage of screening investigations is scarce.

Keywords: Breast Cancer, BSE, Awareness

O-42: Ultrastructural Study of Endometrium in Women with and without Endometriosis

Iroshangar LR*, Soleimani Rad J, Majdi A

University, Embryology and Histology, Tabriz, Iran
Email: lroshangar@yahoo.com

Objective: Endometriosis is the cause of infertility in more than 40% of infertile women. Study of endometrial cellular behavior and their morphology at ultrastructural level will help to knowing the etiology of endometriosis. The aim of the present study is investigate the morphological characteristic of cellular organelles in endometrial epithelial and stromal cell in women with and without endometriosis.
Materials and Methods: In the present case-control study, endometrial specimens from 8 women without endometriosis (as control) and 8 women with endometriosis were examined. The patients were referred to gynecology hospital for various treatments. The specimens were fixed in 2%glutaraldehyde and then post fixed in osmiumtetroxide, embedded in araldite. Semithin sections were stained with tuluidin blue and studied with LM. While, thin sections were prepared and studied with TEM.
Results: The preliminary results show that cells from endometirum of women with endometriosis have euchromatic nucleus in comparison to those without endometriosis. The ultrastructural features of organells were not remarkably different between two group. However the extracellular matrix in the stroma from endometrium of women with endometriosis appeared less dense than those in women without endometriosis.
Conclusion: Regarding the supportive role of stromal cells on luminal and glandular epithelium an exact study of endometrial extracellular matrix is recommended. On the other hand the euchromatic state of nuclei in cells from endometrium of women with endometriosis is suggestive high metabolic activity in those cells.

Keywords: Endometriosis, Endometrioum, Stroma

O-43: Do not Approach Us Like "Lab Rats" or "Numbers": Infertile Women's Expectations of Health Professionals to Meet Their Religious and Spiritual Needs

Latifnejad Roudsari R[1*], Allan HT[2], Smith PA[2]

1. Mashhad University of Medical Sciences, Mashhad, Iran
2. Division of Health & Social Care, Faculty of Health & Medical Sciences, University of Surrey, United Kingdom
Email: r_latifnejad@hotmail.com

Objective: Existing research suggests that religion and spirituality are highly valuable for many people during their confrontation with the fertility crisis. Many patients would like their caregivers to discuss their spiritual beliefs, but it might be difficult for them to bring their religious and spiritual values up in their medical consultations, as they distinguish sacred from secular issues in treatment processes. This study explored infertile women's expectations of health professionals in the context of spiritual care.
Materials and Methods: In this study using a feminist grounded theory approach 30 infertile women affiliated to different denominations of Christianity (10 Protestants, 6 Catholics, 2 Orthodoxies) and Islam (6 Shiites and 6 Sunnis) and also 7 infertile women with no formal religion were interviewed. Volunteer participants were purposively recruited in one Iranian and two UK fertility clinics and the sample size was determined by theoretical sampling and data saturation. Data were collected through semi structured in-depth interviews, post interview notes, research diaries and a quantitative tool entitled Religious Spirituality Assessment Inventory. Data analysis was carried out using Strauss and Corbin's mode of grounded theory by means of Nvivo software, Version 2.
Results: Both religious and non-religious participants expected health professionals to be honest, sincere, understanding and sympathetic. They criticized depersonalization and approaching people like "numbers" and not human beings. Religious participants had extra expectations like addressing their spirituality and undertaking religious rituals before the treatment procedures by the medical team. They expected the establishment of a faith-based doctor-patient relationship which could give them hope and contentment. They believed that addressing religious and spiritual issues by health professionals would be peaceful, encouraging, positive and not only make the experience satisfying and pleasant but also would have long-term impact on their psychological well-being. They also viewed IVF clinics as specific treatment settings which are endeavoring to create human beings and indeed are dealing with people's life. So it is expected that they try to be more compassionate, sensitive and caring and do not approach people like "lab rats".
Conclusion: I argue that the multidisciplinary team who approach infertile women including doctors, midwives, nurses, psychologists and counselors should be encouraged to be attentive to all dimensions of patients and treat them as a whole person with all physical, social, emotional and spiritual needs. They should develop a basic understanding of the patients' religious and spiritual requirements in order to identify the patients who are struggling with these issues.

Keywords: Infertility, Religious and Spiritual Needs, Health Professionals, Feminist Grounded Theory

O-44: Treatment of Uterine Fibroma by Using Herbal Compound (Called F2)

Saeed GSH

Tamine Ejtemaei, Kerman, Iran
Email: dr.reza_saeed@yahoo.com

Objective: Treatment of uterine fibroma has been changed during recent years by introduction of LH and RH (luteinizing hormone releasing hormone). These medicines, which are active via parenteral administration, have proved remarkably effective and, are devoid of metabolic effects. They do engender some disagreeable adverse reaction, and unfortunately their efficacy is transitory and their cost is high.
Materials and Methods: According to traditional treatment which mentioned in Zakhireh Kharazmeshahy (narrated by Jorjany), Exire Azam (), and Avesina Medical Law there are herbal compound (we called F2) which can be used as vaginal suppository has excellent affect on the uterus fibroma.
Results: In this study we applied F2 on eight patients suffering uterus fibroma, with the following results. Five (62.5%) patients were cured and did not have any sign of disease during at least four months. The result of treatment in one (12.5%) patient was mild but in two (25%) other patients remained moderate.
Conclusion: In patient suffering Uterine Fibroma it is recommended to apply the herbal medicine which is cheaper and cure the disease, instead of LH and RH (luteinizing hormone releasing hormone) which are expensive and have transient effect.

Keywords: Herbal Medicine, Uterus, Fibroma

O-45: Immunologic Markers and Immunotherapy Outcome in Patients with Unexplained Infertility, Primary and Secondary Recurrent Pregnancy loss

Sherkat R*, Farzanfar E, Farajzadegan Z, Kalantari A, Ahmadi M, Daneshmand F, Arshadi M, Alameh T, Alameh Z, Movahedie M, Mard anian F, Rezaie A

Isfahan University of Medical Sciences, Isfahan, Iran
Email: sherkat@med.mui.ac.ir

Objective: Whether maternal immune effector mechanisms cause pregnancy loss, and whether effective treatment is possible are subjects of controversy. Combination therapy appears promising, but better diagnosis of subgroups imunological markers and responsive to specific therapies is critical. Several immunological factors have been associated with diagnostic subpopulations of reproductive failure. It is important to determine a trend of immunological abnormalities among these subpopulations

Materials and Methods: patients with Unexplained infertility (UI), Recurrent Primary and secondary Pregnancy loss has evaluated according to two immunologic aspects, namely "autoimmune" and "alloimmune". To compare the frequency of abnormal immunologic tests "serum Antinuclear (ANA) , IgM,IgG Anti-phospholipids (APA), IgM,IgG Anti cardiolipin (ACA), Anti thyroid peroxidase(TPO), Anti-thyroglobulin antibodies (ATA), Lupus anticoagulant, Gamma globulins, Anti paternal cytotoxic antibodies (APCA), CD3, CD4, CD8, CD16+56", among women experiencing reproductive failure ,18 patients with UI, 87 Recurrent primary and 32 secondary abortions were prospectively included. In patients with immunopathological problems, immunotherapy (Aspirin, low molecular weight heparin, Intravenous Immunoglobulin…) depends on their background disorder and our clinic protocol was applied during their next pregnancy.

Results: The frequencies of abnormal immunologic markers were high in all three groups in comparison to normal values. All immunological markers were dramatically high in primary aborters but it wasn't significant statistically .Only anti-ATA positivity was seen equally in primary and secondary aborters , 40%, 46%, perceptively . Whereas, it was 13% positive in infertile group (X2= 7.2 df =2 Pv = 0.02)

The outcome of treatment, as alive birth, in UI, primary and secondary RSA were 100%, 86.2% , 84.4%. The Poor outcome significantly related to APCA negativity (P Value =0.02) low CD3 (P Value =0.006), abnormal CD4 (P Value=0.02) in primary aborters , and low CD8 in UI and RSA patients (P value=0.09) .

Conclusion: A better understanding of how the fetus as an allograft escapes immunological rejection and how cytokines inducing immunologic tolerance to the fetal allograft could have major implications in the diagnosis and treatment of repeated miscarriage and also unexplained infertility. Although genetic, anatomic and hormonal factors have been implicated as the cause of pregnancy loss, a substantial proportion of cases which reminded as unexplained attempted to elucidate the immunological mechanisms and this is also the case for patients with unexplained infertility. Our results show that immunopathological checkup and immunotherapy is useful for the patients with unexplained pregnancy loss . However the success of this method depends on the adherence of the checkup protocol, because unsuccessful therapy of nonclear cases can reduce the efficiency. More clinical trials needed in this area.

O-46: Evaluation of Auto & Alloimmune Factors in Women with Recurrent Abortion

Sherkat R, Kalantari A, Ahmadi M, Hasanzadeh A, Daneshmand F, Arshadi M, Alameh T, Alameh Z, Rezaie A, Baghaie A, Khanjani A, Mardanian F

Isfahan University of Medical Sciences, Isfahan, Iran
Email: sherkat@med.mui.ac.ir

Objectives: The majority of cases of recurrent spontaneous abortion (RSA) remains unexplained and is found to be associated with certain autoimmune (anti phospholipid (APA), (ACA), antinuclear (ANA), anti thyroid (ATA), and anti endothelial cell antibodies and alloimmune(anti paternal cytotoxic antibodies(APCA),…) factors that may play major role in the immunologic failure of pregnancy. Evidence supports the association between infectious agents and the presence of APA's antibodies

Methods and Material: To evaluate immunological background of our patient, in a cross sectional study 142 of the unexplained RSA cases underwent an immunological screening protocol. Serum ANA, APA (IgM, IgG), ACA (IgM, IgG), ATA, Anti-CMV (IgM, IgG), Lupus Anticoagulant, Gammaglobulins, APCA level measured by ELiZA, Electrophoresis and… methods.

Resuls: ANA, APA (IgM, IgG), ACA (IgM, IgG), ATA, Anti-CMV (IgM, IgG), Lupus Anticoagulant, Gammaglobulins, APCA were positive in 21.1%, 26.8%, 16.2%, 23.2%, 19.7%, 12.7%, 11.4%, 80%, 5.6%, 39.4%, 43.7% of cases.A direct relation has been found between APA (IgM, IgG) and ACA (IgM, IgG), r=0.792 p=00 & r=0.215 p=00, also IgM-anti CMV, number of abortions each with gammaglobulins ratio in serum, r=0.270 p=0.008. IgG Anti-CMV had direct relation with consanguinity of couples as well, r=0.239 p=0.06.

Conclusion: Immunological disturbances play a role in majority of patient with RSA.

Different types of anti phospholipid antibodies and higher serum ratio of gammaglobulins with no statistically significant relation with auto and allo antibodies have been noted.

The role of viruses in induction of an autoimmune condition and whether women with unexplained RSA have difficulty in responding to CMV, all needs more future reseaches.

Genetics

O-47: Possible Association Between In Vitro Fertilization Therapy and Cancer Incidence In Women And Their Offspring

Heidari MM, Rezvanifar M*, Tabatabaei V

SBMU,Shaheed Beheshti Medical University, Tehran, Iran
Email: M.Rezvanifar@sbmu.ac.ir

Objective: Infertility is currently a major clinical problem, and a recent report from the World Health Organisation (WHO) suggests that 80 million people are affected worldwide. During the past two decades, assisted reproductive technologies (ARTs) have revolutionised the treatment of infertility and in vitro fertilization (IVF) services are growing worldwide. Since 1978, nearly one million babies have been born worldwide following ART. Over the past decade attention has been increasingly focused on the long-term health effects of ART, such as IVF, in both women and their offspring. There is concern about the long-term health impact of ovarian stimulation treatment for infertility, in particular the effect on cancer risk. Several reports have been published on cancer in children born after IVF or fertility drug use. Unfortunately, many of these publications have been case reports, which cannot constitute proof of a causal relationship. Therefore, this review article assesses the existing literature to determine the risk of cancer both in children conceived by IVF or fertility drug use and in women who underwent it.
Materials and Methods: To identify relevant papers used in this review, a MED-LINE search from January 1990 until March 2008 was performed. We searched for all original, review, and case report English-language papers that examined infertility treatment in relation to the risk of cancer both in women who underwent IVF or fertility drug use and their offspring. Additional papers were added by examining references of overview articles in the relevant fields.
Results: We found some studies about potential risk of certain cancers in women and in children conceived by IVF or fertility drug use. The women cancers include Melanoma, Thyroid, Ovarian, Endometrial, Cervical, Breast and Colon cancer. Neuroblastoma, Retinoblastoma, Haematological malignancies (mainly leukaemias), and Tumours induced by imprinted genes were reported in children conceived by IVF or using fertility drugs.
Conclusion: To our knowledge, we found no evidence that ART, or more specifically IVF treatment, is associated with an increased risk of cancer in children. It is possible that the drugs and procedures involved in ART may lead to epigenetic modification of DNA and alter imprinted gene expression, potentially resulting in the development of cancer in the offspring. It appears that IVF therapy might increase the risk of some women cancers.

Keywords: Assisted Reproductive Technology, IVF, Cancer, Children Health, Fertility Drugs

O-48: Structural, Functional and Molecular Aspects of Follicle Stimulating Hormone Receptor: Applications in Designing Receptor Targets and Management of Female Infertility

Mahale SD*, Ghosalkar JD

Nat Inst Res Repro Health, Structural Biology, Mumbai, India
Email: smitamahale@hotmail.com

Objective: Follicle stimulating hormone (FSH) acts through specific receptors present on the target cells in the gonads. Extracellular domain (ECD) of the follicle stimulating hormone receptor (FSHR) plays an important role in ligand binding and ligand specificity. The objectives of our project are (i) to identify regions in the receptor ECD, involved in hormone binding and signal transduction; (ii) to identify bioneutralizing epitopes of FSHR; and (iii) to study FSHR genotype with an aim to develop a molecular method for predicting ovarian response in an ART programme.
Materials and Methods: Series of peptides from the ECD were synthesized by solid phase peptide synthesis technology. Antipeptide antibodies were developed to these peptides after conjugating to carrier protein. Effect of peptides and antibodies on binding of the hormone to the receptor was tested by radioreceptor assay. Effect on signal transduction was studied by measuring FSH induced cAMP levels. HEK 293 cells expressing FSHR were used as receptor source. Bioneutralization of FSHR activity in vivo was tested in a female rat model, where the effect of passive administration of antibodies on pregnancy outcome was monitored. Single nucleotide polymorphisms in FSHR gene were studied in females undergoing IVF/ET programme and genotype-phenotype correlation was done.
Results: Peptides from the C-terminal part of FSHR were effective in inhibiting hormone binding and signal transduction. A small peptide 20-30 was also effective at much lower concentrations and the inhibitory effect on FSH binding was more than 90%. Antibodies to peptide 285-309 were able to recognize parent receptor in western blot and also FSHR in the rat ovary. These antibodies also inhibited receptor activity both in-vitro and in-vivo.
Conclusion: Peptide 20-30 acts like a potent FSH antagonist and this information will be helpful in the design of molecules in the development of FSH regulating agents. Antibodied to peptide 285-309 neutralized the biological activity of endogenous receptor, which resulted in the induction of infertility in the treated animals. Thus bioneutralization of FSHR was achieved by targeting the region 285-309 of FSHR in an in-vivo system. Molecular studies performed in Indian women revealed that FSHR polymorphism (Thr307Ala) is associated with variable ovarian response and ovarian hyperstimulation syndrome.

Keywords: FSH, FSHR, Bioneutralizing Epitope, FSHR Polymorphism

O-49: Chromatin Abnormality and DNA Damage Recognition in the Rat Cauda Epididymal Sperm Following Chronic Paternal Cyclophosphamide Exposure: the Protective Role of Satureja Khuzestanica Essential Oil as a Potent Natural Antioxidant

Rezvanfar MA[1*], Sadrkhanlou RA[1], Ahmadi A[1], Rezvanfar A[2], Salehnia AN[2], Abdollahi M[2]

1. Laboratory of Embryology, Department of Embryology and Histology, Faculty of Veterinary Medicine, Urmia University, Iran
2. Laboratory of Toxicology, Department of Toxicology and Pharmacology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Iran
Email: amin2_dvm80@yahoo.com

Objective: Treatment with cyclophosphamide (CP), a commonly used anticancer and immunosuppressive agent, may result in oligospermia and azoospermia. CP administration induces oxidative stress and is cytotoxic to normal cells. It is hypothesized that CP exerts its effects by targeting specific components of spermatozoal nuclei. In this context, we have studied the effect of an established natural antioxidant, Satureja khuzestanica Essential Oil (SKEO) on CP-induced oxidative DNA injury in rat sperm.
Materials and Methods: Male Wistar rats of 220-230 g were categorized into four groups. Two groups of rats were administered CP (6 mg/kg body weight once a day for 28 days by oral gavage); one of these groups received SKEO treatment (225 mg/kg body weight by oral gavage once a day for 28 days; 4 h prior to CP administration). A vehicle treated control group and a SKEO control group were also included. To evaluate sperm chromatin quality and DNA integrity, Aniline blue and Acridine orange staining were done in all of groups. The plasma samples were separated and kept at -80°C until analysis of oxidative stress markers (levels of lipid peroxidation and total antioxidant power). In addition, one testis kept frozen at -80°C until homogenized for further analyses of oxidative stress biomarkers. FRAP and TBARS assays were used to determine total antioxidant power and lipid peroxidation respectively.
Results: The testis and plasma of untreated CP-exposed rats showed a significant increase in lipid peroxidation, along with a significant decrease in total antioxidant level. These changes were associated with significant increase in DNA damage and chromatin abnormality in the cauda epididymal sperm as evidenced by acridine orange and aniline blue staining respectively.
Conclusion: Pretreatment with SKEO significantly reduced the oxidative stress, DNA damage and chromatin abnormality induced by CP, thereby demonstrating the protection rendered by SKEO.

Keywords: Cyclophosphamide, Satureja Khuzestanica, Sperm, DNA Damage, Lipid Peroxidation, Oxidative Stress

O-50: Simulaneous Fetal Cell Detection and Genetic Diagnosis by Immunophenotyping and Chromosomal Fluorescence in Situ Hybridization

Sitar G

Clinica Medica 3°, Policlinico San Matteo, 27100, PAVIA, Italy
Email: g.sitar@smatteo.pv.it

The isolation of fetal cells from maternal blood for prenatal non-invasive genetic investigation is presently in progress in many laboratories worldwide and several procedures have been described, although a routine clinical test is not yet available.
Whichever procedure will eventually be the most successful, the limited number of fetal cells available for genetic analysis will represent a technical challenge or a limiting factor for routine investigation. Interphase cytogenetic by FISH is one possible approach, between several, to genetic investigation of fetal cells isolated from maternal blood. This approach has two major limitations, first target cells are distributed between a background of a large number of maternal cells, FISH analysis therefore results in a tedious and error-prone procedure in which fluorescent spot-like signals have to be scored in hundred of thousands of cells while constantly changing the plane of focus in order not to miss signals from out-of-focus planes. An automatic FISH scoring system would represent a major improvement. Secondly an unresolved difficulty with this approach is the inability to distinguish a fetal cell unequivocally before the analysis of its chromosome constitution. Both these inconveniences would be solved by combining immunocytochemistry (ICC) evaluation with fetal cell in situ hybridization (FISH) through a specific fetal-cell marker.
The search for an antibody that recognizes an antigen that is unique to all fetal cells present in maternal blood has been so far unsuccessful. Simultaneous ICC and FISH, in prenatal non-invasive genetic investigation, has been described so far only with anti-γ and anti-ε globin chains-Hb monoclonal antibodies which recognize only fetal erythroblasts while entirely missing fetal CD34 stem cells, which are also represented in maternal blood.
Which fetal cell type is more largely represented in maternal blood is so far unknown. Philip and his group have provocatively suggested that "most fetal cells found in maternal blood by FISH methods may not be NRBCs". An interesting alternative to embryonic and fetal-Hb antibodies might be a monoclonal antibody for i-antigen since in adult female control cells i-positive cells are very rare. This antigen is formed by a straight oligosaccharide chain of N-acetyllactosamine subunits and is not confined to human erythroid cells, being present on several cell types, and predominates on fetal cells early and late in pregnancy, the switch to I-antigen happens after birth therefore it is suitable for investigation in maternal blood early and late in pregnancy while embryonic and fetal-Hb-chains are continuously switching during pregnancy.
In this report we investigate several different procedures for the simultaneous detection of i-antigen and sex chromosome identification.

Stem Cells

O-51: Label Free Optical Technique to Monitor Stem-Cell Substartum Interactions

Aref A[1*], Horvath R[1], McColl J[2]

1. Cranfield University, Nanotechnology, Cranfield, England
2. University of Cambridge,Chemistry,Cambridge, England
Email: a.aref@cranfield.ac.uk

Objective: In this paper we have investigated the attachment and spreading kinetics of human embryonal carcinoma stem cells (TERA2.sp12) onto planar Si(Ti) O2 waveguides equipped with grating couples, and covered with poly-L-lysine (PLL), mucin acting as substrata for the cells. Analysis of the incoupling peak centre and width allows us to separate surface-molecular and bulk-cell effects of the three substrata investigated. The most extensive spreading is observed on PLL, coating with mucin completely inhibits cell spreading. We have also demonstrated the utility of OWLS for the quantitatives label-free investigation of stem cell attachment and spreading.
Materials and Methods: Cell culture & biochemicals Human EC cell lines, TERA2, were generously provided by S. Przyborski, Durham University, UK. Pharmaceutical grade porcine gastric mucin (PGM) was purchased from A/S Orthana Kemisk Fabrik, Kastrup, Denmark. The commercial mucin preparation with a mean molecular weight estimated as 565 kDa was dialysed to remove all salts and low molecular weight additives and lyophilized for storage. Substratum modification Waveguides were cleaned at room temperature with chromic acid (Fisher Scientific, U.K.) or under sonication in Colas Integra (Roche) cleaning solution or SDS/Colas Integra solution for 10 minutes, extensively rinsed using Elga ultrapure water (resistance 18.2 Mohm cm, filtered through 200 nm pores) and O2-plasma treated (20 mW for 2 min). The PLL (0.01% solution; Sigma) and mucin (0.1% w/w) stock solution were made up by dissolving weighted dry material in ultra pure water this is sufficient to coat the waveguides and pre-equilibrated overnight. Solutions were applied to the waveguides for 20 minutes, washed twice with ultrapure water and incubated for at least 40 minutes in cell culture media (DMEM; high glucose (4500 mg/L); pyridoxine HCl; NaHCO3; without L-glutamine) (Sigma) at 37 oC, 5% CO2 environment. Cell attachment Cells were detached from the culture flask using 0.05% trypsin/EDTA and collected using centrifugation (1500 rpm for 3 minutes). Cells were counted by eye using a hemocytometer, with the number of cells and surface coverage (60-70%) confirmed using phase contrast microscopy inspection of the waveguide after the experiments. Optical waveguide lightmode spectroscopy Waveguides were made from amorphous silica:titania at a ratio of approximately 2:1 and incorporated a shallow (5-10 nm) grating coupler (type 2400, grating constant equal to 416.667 nm). The incoupling resonance peaks for the TM0 mode of the waveguides were measured every 40 seconds and saved for subsequent analysis.
Results: The peak position is influenced by the presence of the cells in close contact with the substrata, their shape, and any protein exudates from the cells, which are deposited on the substratum. The two effects can be separated by considering simultaneously the surface coverage of the cells influencing the shape of the peaks. As explained above the appearance of a maximum in the plot of the overall width against cell development magnifies 50% cell coverage. The actual magnitude of the peak width depends on the optical contrast between the uncovered and cell covered areas. The larger the cells, or the more tightly they are bound, the larger the contrast. We can exclude the first possibility since the cell numbers were identical. Therefore we infer that the cell bind about 4 times more strongly to the PLL than to the silica-titania. Finally we turn to mucin. Although there is a modest peak position shift, there is a total absence of peak broadening – implying no spreading (corroborated by the optical micrographs . Therefore these type of stem cells most probably just exclude.
Conclusion: Simultaneous measurement of incoupling peak position and width allowed us to get more detailed information about the surface behavior of living stem cells then previously demonstrated using only peak position data. We found that mucin totally inhibits the spreading of human embryonal carcinoma (TERA2) stem cells when compared with uncoated silica-titania, but the cells still register some physiological activity, while on the PLL surface cell spreading dominated the optical signals.

Keywords: OWLS, Human Embryonal Carcinoma Stem Cells, Attachment, Spreading, Cell Substrata Interaction

O-52: The Molecular Mechanisms Controlling Embryonic Stem Cells (ESCs) Proliferation and Differentiation

Fathi A[1], Pakzad M[1], Salekdeh GH[2]

1. Cell Sciences Research Center, Royan Institite, ACECR, Tehralsfahan, Iran
2. ABRII, Physiology and Proteomics, Karaj, Iran
Email: a_fathi2000@yahoo.com

Objective: Over the past few years, there has been a growing interest in discovering the molecular mechanisms controlling embryonic stem cells (ESCs) proliferation and differentiation. Proteomics and transcripteomics analysis showed to be an effective approach to comprehensively unravel the regulatory network of differentiation
Materials and Methods: We applied a two dimensional electrophoresis based proteomic approach followed by mass spectrometry to analyze the proteome of two Human ESC lines, Royan H5 and Royan H6, at 0, 3, 6, 12 and 20 days after differentiation initiation and the transcripteom analysis performed by microarray DNA chips for a total of 20597 genes
Results: Out of 127 differentiation associated proteins

detected in two lines, 35 proteins were common. Mass spectrometry analysis of these protein spots led to identification of 92 proteins. Our results showed that proteins involved in signal transduction, Metabolism, cell motility and Transport are the main proteins that differentially expressed whereas Immune response and stress related proteins have a less abundance related to total differentially expressed proteins. Proliferation associated proteins such EBP1 (Erbb1 binding protein), RCL (putative c-myc responsive protein), Nucleophosmin (Multifunctional protein) and HSC70 tested by western blotting and immunocytochemistry. Concurrently transcripteomics analysis with microarray and real-time quantitative PCR approaches for candidate proteins are running.
Conclusion: Several novel ESC-associated genes and proteins have been presented in this study which warrants further investigation with respect to the etiology of stemness.

Keywords: Human Embryonic Stem Cells, Proteomics, Transcripteome

O-53: Endogenous Adult Neural Stem Cells Comprise the Minority of Label Retaining Cells in the Adult Mouse Brain

Golmohammadi M[1*], Golmohammadi M[2], Azari H[3], Mardani M[4], Esfandiari E[4], Rietze L[5]

1. A.U of Medical Sciences, Anatomy, Ardebil, Iran
2. School of Medicine-Ardebil, Anatomy, Ardebil, Iran
3. Medical School-Shiraz, Anatomy, Shiraz, Iran
4. Medical School-Esfahan, Anatomy, Isfahan, Iran
5. Queensland Brain Institute, QBI, Brisbane, Australia
Email: golmohammadi50@gmail.com

Objective: Given rapidly dividing and slow cycling populations of neural precursors have been identified in the adult brain , with several lines of evidence suggesting the latter population represents Neural Stem Cells (NSCs) ,we hypothesized that adult NSCs could be identified using Label Retaining Cell (LRC) approach.
Materials and Methods: Adult CBA mice were injected with the mitotic marker bromodeoxyuridine (BrdU), every two hours for 48 hours. We compared the number of LRCs cells detected in a 400um region of the PVR at increasing chase periods, to the number of primary neurospheres and NSC-derived colonies (Large Colonies) that could be generated from the same regionusing Neurosphere Assay (NSA) and Neural Colony-Forming cell Assay (N_CFCA).
Results: While the prevalence of LRCs and neurospheres and overall colonies was equivalent at specific chase periods, the number of NSC-derived colonies remained reduced by at least two orders of magnitude for chase periods up to 7 months.
Conclusion: Our results suggest that < 5% of LRCs are bona fide Neural Stem Cells, and highlights the pitfalls of employing this methodology to discern stem from progenitor cell populations.

Keywords: Neural Stem Cells-Mouse-Label Retaining Cell

O-54: Adenosine A2A Receptor Play an Active Role in Mouse Bone Marrow Stromal Cell Proliferation and Differentiation to Mesenchymal Stem Cells

Katebi M

New York University, Medicine, New York, USA
Email: majid@gmail.com

Objective: Cellular therapy is gaining interest as a means of improving the prognosis of patients with failure of various organs. Numerous in vivo and in vitro studies have shown the potential of stem cells in the regeneration of organs such as heart, muscle, cartilage, and tendon; these cells are therefore used in tissue engineering. Adult bone marrow contains a minority population of Mesenchymal Stem Cells (MSCs) that contribute to tissue regeneration. Previous studies in our lab. have demonstrated that A2A receptor accelerate wound healing and tissue repair. MSCs express SH3 and SH4, two distinct epitopes of the ecto-5-'-nucleotidase (CD73), the enzyme that produces adenosine. The aim of this study was to determine whether A2A receptor may also modulate MSCs proliferation and differentiation.
Materials ans Methods: Bone Marrow MSCs were isolated and cultured from A2A deficient female C57Bl/6 mice. We also used cells from CD73 deficient female C57Bl/6 In mRNA studies. Adenosine receptor and CD73 expression was analyzed using PCR and Real Time PCR. We identified and quantified MSCs among the adherent cells cultured by colony forming Unit-Fibroblastic (CFU-F) assay. Procollagen α2 Type I expression was determined by western blotting and immunocytochemistry. In an effort to understand how A2A receptor control MSC properties, we focused on determining stem cell specific markers. To study Phenotypic characterization, cells from primary culture and third passage, labeled with the appropriate dilution of one of the following antibodies: anti mouse Procollagen α2 Type I , CD11b, CD34, CD44, CD45, CD73 (SH3-SH4) ,CD90 (Thy-1),CD105 (TGF-B Co-receptor also named SH2 or Endoglin), CD133. PCR and RT-PCR utilized to study adenosine receptor expression.
Results: Adherent cells cultured from Bone Marrow population showed diverse morphologies. More homogenous cell population was obtained after Subcultures. We observed that CFU-F numbers, obtained at day 12 is reduced significantly in both BM MSCs cultures from A2A receptor knockout mice and A2A receptor antagonist (ZM) treated Cells as control. The selective adenosine A2A receptor agonist, CGS 21680, fails to induce a significant increase in number of colonies. Western blotting and Semi-quantification of Procollagen α2 Type I immu-

nocytochemistry showed decrease collagen expression in A2AKO and ZM 10-6 M treated cells. Our results showed the cells at third passage are positive to Cd11b, CD45, CD44, CD73, CD90, CD105 and Procollagen Type I but negative or weak expression of CD34 and CD133. Our results show that significantly fewer and CD90, CD105and Procollagen Type I ($p<0.05$) were observed in A2AKO cells than were observed in A2AWT cells as control. Semi-quantitative PCR detects CD73 and all adenosine receptors, A2A, A2B, A1, and A3 in third passage BM-MSCs. Relative quantitative PCR Results suggests a correlation between CD73 mRNA level and A2A expression and Vice versa. We found mRNA level of A2A receptor decreased in CD73 deficient mouse and also CD73 mRNA level decreased in A2A deficient MSCs.
Conclusion: These findings indicate critical role of A2A receptor in proliferation and differentiation of mouse BM-MSCS. Our results are first evidence of role of A2A receptor in CD90 (Thy-1) and CD105 expression in Stem Cells. We also found that CD73 and A2A receptor can regulate their engagement in MSCs.

Keywords: Mesenchymal, Stem Cells, Adenosine, Receptor, A2A

O-55: Intra-Epidermal Transplantation of Autlogous Melanocyts Improved Pigmenation of Vitiligo Patients

Khodadadi L*, Shafieyan S, Aghdami N, Baharvand H

Cell Sciences Research Center, Royan Institite, ACECR, Tehran, Iran
Email: laleh_khodadadi@hotmail.com

Objective: Autologous transplantation of melanocytes in vitiligo patients by suction blister, laser and dermabrasion to recipient site(s) has at least few limitations such as activation of köbner phenomen in recipient area, requirement to special equipments and then relative higher cost and convalescence period. In this study we evaluated the safety and effectiveness of intra-epidermal melanocyte injection into lesions of vitiligo patients.
Materials and Methods: Ten stable vitiligo patients (four male, six female) with mean age 29.6 (range 17-52 years) and mean disease duration 8.65 years were included in this study. To isolate single epidermal cells including melanocytes, skin biopsies were treated by dispase to remove epidermal layer and then digested by Trypsin/EDTA. Isolated cells from a shave biopsy skin sample up to 1/5th the size of recipient were injected intraepidermally. It is notable that any of patients did not receive other adjuvant therapy such as UVA or cryotherapy. The response was evaluated as marked (76-100%), moderate (51-75%), mild (26-50%), minimal repigmentation (1-25%) and/or no response.
Results: Repigmentation started during 4 weeks after transplantation in all cases and marked repigmentation was seen in patients who completed their 4 months follow up. The start time of pigmentation was different based on cell count (2 to 4 weeks); however the correlation between start of pigmentation and the disease duration was weak ($R2= 0.4$). No side effects were observed in any of the patients. Interestingly, the repigmentation of gray hair in one patient after 4 month post transplantation was observed
Conclusion: Intra-epidermal transplantation of autologous melanocytes is an effective, simple and safe therapeutic option for stable vitiligo lesions. As the patients did not receive other adjuvant therapy andalso for no discoloration, minimal convalescence period and lower cost of this method we can conclude that it is cosmetically more acceptable.

O-56: Enrichment of Undifferentiated Embryonic Stem Cells on a Culture Surface with Glucose-displayed Dendrimer

Mashayekhan S[1*], Miyazaki JI[1], Taya M[2]

1. Osaka University, Medical School,Osaka, Japan
2. Osaka University, Chemical Engineering, Osaka, Japan
Email: smashayekhan@gmail.com

Objective: Embryonic stem (ES) cells have a great interest for tissue engineering because of their pluripotent nature and proliferative capacity. Up till now there have been several reports showing colonization and maintenance of ES cells in undifferentiated state using various biomaterials which are mostly focused on increasing the colonization rate or large-scale expansion of ES cells. In the current study, we developed a serial passaging protocol, which allowed for enrichment of ES cells undifferentiated state cultured on D-glucose-displayed dendrimer surface.
Materials and Methods: The murine ES cell line EB3 was maintained in Glasgow Minimum Essential Medium (GMEM) with 1000 U/ml leukemia inhibitory factor (LIF). For passaging the cells on D-glucose-displayed dendrimer, the loosely attached spherical colonies were harvested by tapping at day 4, dissociated into single cells using Trypsin/EDTA, and then inoculated again into subsequent culture systems. Following this same procedure every 4 days, ES cells were carried through four passages for a total of 16 days of culture. ES cells cultured on gelatinized surface, which were run simultaneously, went through over the same 16 days.
Results: The results of colony morphology observation at day 4 revealed that the cells on D-glucose-displayed surface could form loosely attached spherical shape colonies while the ES cells cultured on the conventional gelatin-coated surface formed flatter colonies, firmly attached to the surface. The possibility of using dendrimer surface as a tool for enrichment of undifferentiated state of ES cells was investigated by culturing the cells on D-glucose-displayed dendrimer as well as gelatinized surface for several passages. The undifferentiated state of cultured cells on different surfaces was compared by both alkaline phos-

phatase (ALP) staining and RT-PCR analysis. The results showed that ALP activity of the spherical colony cells on D-glucose-displayed dendrimer increased with increasing the cell passage number. The result of RT-PCR analysis showed that undifferentiation cell markers (Rex-1 and Oct3/4) were detected at higher levels for spherical colony cells on D-glucose-displayed dendrimer surface comparing to the cells grown on gelatinized surfaces. Further, in the case of early differentiaition markers, the results of RT-PCR revealed that markers for early endodermal (GATA4), mesendodermal (Gsc) and mesodermal (T, Wnt3) differentiation were expressed at lower levels for spherical colony cells on D-glucose-displayed surface comparing to the cells grown on gelatinized surfaces. Therefore it can be suggested that by using the mentioned serial passaging protocol, we can enrich the undifferentiated state of ES cells cultured on D-glucose-displayed surface.
Conclusion: In the present study we demonstrated that mouse ES cells cultured on a D-glucose-displayed surface could be maintained as an ES cell population with a unique colonial morphology, and that these cells showed a greater tendency to retain undifferentiated characteristics than those grown under conventional conditions. The applied culture surface may be a useful alternative tool for obtaining cell preparations enriched in ES cells in an undifferentiated state for the future cell therapy applications.

Keywords: Embryonic Stem Cells, Enrichment of Undifferentiated Cells, Glucose-Displaying Dendrimer Surface

O-57: Inducible RNA Interference in Embryonic Stem Cells to Study Transcriptional and Posttranscriptional Regulators of Differentiation

Moroni C

Microbiology, Biomedicine, Basel, Switzerland
Email: christoph.moroni@unibas.ch

Objective: Although differentiation of pluripotent embryonic stem cells is restricted by a hierarchy of transcription factors, little is known about whether post-transcriptional mechanisms similarly regulate early embryoid differentiation. We wished to develop a system where small hairpin (sh)RNAs can be induced in embryonic stem (ES) cells in order to downregulate candidate genes of development.
Materials and Methods: We introduced a flp recombination target into a defined locus marked by the gfp gene a defined locus. This allowed controlled insertion by Flp mediated DNA recombination following transfection of flipase an frt-marked shRNA fragment. The cell was engeneered to allow induction of shRNA expression following doxycyclin treatment (Tet system). We introduced shRNA specific for the transcription factor Stat3 and the RNA-binding protein Brf1.
Results: Downregulation of Stat3 led to vigorous differentiation inspite of the presence of LIF, as observed morphologically an confirmed by marker analysis. Downregulation of Brf1 led, unexpectedly, to induction of cardiac markers and to enhanced formation of beating bodies.
Conclusion: These findings identify Brf1 as a novel potential regulator of cardiomyocyte formation and suggest that post-transcriptional mechanisms are of importance to early development and, possibly, to regenerative medicine. The inducible RNA interference system presented here should also allow assignment of function for candidate genes with suspected roles in ES cell development.

Keywords: RNA Interference, Stat3, Brf1, Cardiomyogenesis

O-58: Functional Similarities Among Genes Regulated by OCT4 in Human Mesenchymal and Embryonic Stem Cells

Rameshwar P

New Jersey Medical School, Medicine- Hematology/Oncology, Newark, USA
Email: rameshwa@umdnj.edu

Objective: OCT4 is a master transcriptional regulator, which mediates pluripotency in embryonic stem cells (ESCs) through inhibition of tissue-specific and promotion of stem cell-specific genes. Suppression of OCT4, along with other regulators of pluripotency, such as SOX2 and NANOG, has been correlated with cell-fate specification and lineage-specific differentiation. Mesenchymal Stem Cells (MSCs) are mesoderm-derived cells, primarily resident in adult bone marrow (BM), which undergo lineage-specific differentiation to generate specialized cells such as stroma, fat, bone and cartilage. The objective is to determine if OCT4 provides similar regulatory circuitries in human MSCs and human ESCs.
Materials and Methods: The method used ChIP-DSL technology and loss of functional assays with specific si RNA.
Results: The results showed OCT4 providing similar regulatory circuitries in human MSCs and ESCs MSCs were found to express the embryonic transcription factors OCT4, NANOG and SOX2. In addition, OCT4 was found to: (1) target similar genes in MSCs and ESCs; (2) promote the expression of MSC-specific genes; and (3) regulate MSC cell cycle progression.
Conclusion: We concluded that similar regulatory mechanisms exists for the role of OCT4 in MSCs and ESCs. The findings have implications regarding MSC plasticity.

Keywords: OCT4, Adult Stem Cells, Mesenchymal Stem Cells, Eembryonic Sem Cells

O-59: Selective Targeting of Adenoviral Vectors to Neural Precursor Cells in the Hippocampus of Adult Mice: New Prospects for In Situ Gene Therapy

Schmidt A[1], Haas SJP[2], Eckhoff B[2], Wree A[2], Pützer

BM[3*], Racek T[3], Hildebrandt S[3], Scheibe J[3], Kempermann G[4]

1. Biomedical Reserach Center,VEGT, Rostock, Germany
2. University of Rostock, Anatomy, Rostock, Germany
3. University of Rostock,VEGT, Rostock, Germany
4. Charite, MDC, Berlin, Germany
Email: brigitte.putzer@med.uni-rostock.de

Objective: The adult brain contains neural precursor cells (NPC) that are attracted to brain lesions, such as areas of neurodegeneration, ischemia and cancer. This suggests that NPC engineered to promote lineage-specific differentiation or to express therapeutic genes might become a valuable tool for restorative cell therapy and for targeting therapeutic genes to diseased brain regions.
Materials and Methods: Here we report the identification of NPC specific ligands from phage display peptide libraries.
Results: We show their potential to selectively direct adenovirus mediated gene transfer to NPC in adult mice. Identified peptides mediated specific virus binding and internalization to cultured neurospheres. Importantly, peptide-mediated adenoviral vector infection was restricted to precursor cells in the hippocampal dentate gyrus of pNestin-GFP transgenic or C57BL/6 mice.
Conclusion: Our approach represents a novel way for specific manipulation of NPC in the adult brain and may have major implications for the use of precursor cells as therapeutic delivery vehicles in the central nervous system.

Keywords: Neural Precursor/Stem Cells, Gene Therapy, Dentate Gyrus, Adenoviral Vectors, Phage Display, pNestin-GFP Transgenic Mice

O-60: Differentiation and Enrichment of Hepatocypte-Like Cells from Human Embryonic Stem Cells In Vitro and In Vivo

Zern Mark A

UC Davis Medical Center, IM: Gastroenterology, Sacramento USA
Email: mark.zern@ucdmc.ucdavis.edu

Objective: Human embryonic stem cells (hESC) may provide a cell source of functional hepatocytes for therapeutic use. The aim of this study is to develop viable hepatocytes from hESC that can be used for cell-based therapies.
Materials and Methods: Using a combinatorial approach, we developed culture conditions that differentiated a percentage of hESC along a hepatocyte lineage. The differentiated hESC were further enriched by transducing with a lentiviral vector containing the human a1-antitrypsin (α1-AT) promoter driving the GFP gene. The GFP+ hESC were then purified by laser microdissection and pressure catapulting.
Results: After transduction with a lentivirus containing α1-AT promoter, differentiated GFP+ hESC expressed a large series of liver proteins: -fetoprotein (AFP), albumin (ALB), α1-AT, CK18, transferrin (TF), tyrosin aminotransferase (TAT), arginase (ARG), glucose-6-phosphatase, CYP1A1, CYP2B6, CYP1B1, CYP2E1, CYP2C9, and CYP3A. AFP expression decreased with time. Differentiated hESC also had liver-specific functions. Differentiated hESC also had liver-specific functions. They accumulated glycogen, showed the cellular uptake of indocyanine green, and expressed high levels of CYP1A2 activity as well as urea synthesis. Quantitative RT-PCR revealed that the expression levels of purified hESC over time was comparable to primary human hepatoctes: 24 to 35% for ALB, 44 to 57% for α1-AT, 73 to 91% for TF, 13 to 61% for TAT, and 128 to 230% for ARG. When NOD-SCID mice were transplanted with the differentiated hESC transduced with a lentiviral triple fusion vector, positive luciferase signals were obtained by a charged coupled device camera over time. The differentiated hESC survived and engrafted in mouse livers, and human mRNA and protein species (ALB, α1-AT, TF, CYP1A1 and GAPDH) were expressed in the transplanted mouse liver at three weeks after transplantation, and human albumin was detected in the mouse serum. Of note, human AFP expression was not found, indicating that the cells had expression profiles consistent with mature human hepatocytes.
Conclusion: Our results demonstrated for the first time that purified differentiated hESC expressed near physiological levels of liver-specific genes and had liver-specific functions that are comparable to primary human hepatocytes. In addition, this represents the first successful transplantation of differentiated hESC into an animal liver, and the first bioluminescence imaging of hESC in the liver.

Keywords: Hepatocypte, Guman Embryonic Stem Cells, Lentivirus

Poster Presentations

Andrology

P-1: The Influence of Occupational Exposure on Male Infertility

Heidari MM.*, Rezvanifar M.

SBMU, Shaheed Beheshti Medical University, Tehran, Iran
Email: m.rezvanifar@sbmu.ac.ir

Objective: In the last 50 years a significant decrease in human fertility has been observed. Infertility is a common problem affecting one in six couples. The World Health Organization defined infertility as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse for a period of greater than 12 months. In 30% of infertile couples, the male factor is a major cause. There is accumulating evidence that occupational exposures contribute to male infertility. Men suffering from infertility problems may do well to look at their occupations, where exposure to certain substances and situations may be a contributory factor, if not a direct cause, of infertility. The purpose of this review article was to determine the association between male occupational exposures and infertility.
Materials and Methods: The studies examined by the review include those published in the international scientific literature since 1990, and were identified through the search of MEDLINE using selected keywords.
Results: Occupational factors have been divided into 5 groups: 1-Physical factors (Heat, Radiation, Noise), 2-Chemical factors (Smoking, Endocrine disruptors, Solvents, Drugs, Pesticides, etc.), 3-Psychological factors (Psychological disturbances and emotional stresses, Irregular work hours), 4- Exposure to Metals and Welding (Cadmium, Mercury, Chromium, Lead, Nickel, Copper, Manganese,etc.), 5-Physical load and Ergonomic factors
Conclusion: Several occupational exposures have known or suspected deleterious actions to male reproductive function. For some specific agents, such as heat, ionizing radiation etc. the evidence is strongly supported in well-designed epidemiological studies. Additional studies need to be done to ascertain the effects of occupational factors on male infertility. Until then, men and their employers should work together to minimize exposure to these factors.

Keywords: Male Infertility, Occupational Exposure, Fertility, Occupational Factors

P-2: Comparative Study of the Effect of FSH on the Gonadal Physiology of Syrian Mice in the Absence of LH

Rezaei-Zarchi S[1*], Javed A[2], Javeed Ghani M[3]

1. Payam-e-Noor University, Biology Department, Yazd, Iran
2. University of Agriculture, Biochemistry Department, Faislabad, Pakistan
3. Government College University, Bioinformatics Department, Faisalabad, Pakistan
Email: libraforever_2006@yahoo.com

Objective: Gonadal function is wholly dependent on FSH and LH. Identifying the specific effects of the present study was designed to characterize the definitive actions of FSH alone, in the absence of LH effects, created by combining transgenic FSH expression with the gonadotropin-deficient background of the hypogonadal (hpg) Syrian mouse.
Materials and Methods: A tandem transgene was construct encoding each α- and β-subunit of rhFSH, under the insulin II promoter, expressed biologically active hetero-dimers at serum levels, by immunoassay, equivalent to circulating FSH concentrations in fertile humans (0.1–25 IU/l).
Results: Transgenic Syrian mice were crossed into the hpg mouse genotype to obtain LH-deficient animals secreting FSH alone. Testis weights of adult FSH×hpg mice were increased up to 5-fold, relative to nontransgenic hpg controls ($P<0.001$). However, only transgenic males with serum FSH levels more than 1 IU/l showed testis weights increased relative to hpg controls, indicating a physiological FSH threshold for the testicular response. Histology of enlarged FSH×hpg testes revealed round spermatids and sparse numbers of elongated spermatids, demonstrating that the testosterone-independent FSH response targeting the Sertoli cell can facilitate completion of meiosis and minimal initiation, but not completion, of spermiogenesis. Transgenic FSH also induced inhibin B secretion in FSH×hpg mice, but showed a distinct sexual dimorphism with only females exhibiting a strong FSH dose-dependent increase in serum inhibin B levels (r2 5 0.84). In addition, ovaries of FSH×hpg females were enlarged up to 12-fold ($P<0.001$), characterized by increased follicular recruitment and development to type 7 antral follicles.
Conclusion: Thus, these findings show that the transgenic FSH×hpg Syrian mouse provides a unique model for detailed investigations of the definitive in vivo actions of FSH alone.

Keywords: FSH, hpg, LH, Characterization, Spermatids, Gonad

P-3: Effects of Fibroblast Growth Factor (FGF) and Somatomedin-C On the In Vitro Regulation of Syrian Mice Sertoli Cell Growth and Function

Rezaei-Zarchi S[1*], Javed A[2], Ghani MJ[3]

1. Payam-e-Noor University, Biology, Yazd, Iran
2. University of Agriculture, Biochemistry, Faisalabad, Pakistan
3. Government College University, Bioinformatics, Faisalabad, Pakistan
Email: srezaei@ibb.ut.ac.ir

Objective: The effects of insulin, somatomedin-C (Sm-C), epidermal growth factor (EGF), fibroblast growth factor (FGF), vitamin E, and retinoic acid on growth and function of immature cultured Syrian mice Sertoli cells were investigated.
Materials and Methods: Syrian mice streoli cells were exposed to vitamin E, EGF, FGF and retinoic acid for 6 hours.
Results: All these factors, except vitamin E, stimulated Sertoli cell DNA synthesis and proliferation. The mitogenic effects of insulin observed only at micromolar concentrations were similar to those induced by nanomolar concentrations of Sm-C or EGF, but significantly less than those induced by FGF. The effects of EGF and Sm-C were almost additive, whereas those of Sm-C and FGF were synergistic. After a 6-day treatment, FGF and retinoic acid induced a significant increase in the number of follicle-stimulating hormone (FSH) receptors per cell, and in FSH-induced cyclic adenosine 3′, S′-monophosphate (cAMP) production. Sm-C, which alone had no effect on these two parameters, potentiated FGF action. Basal plasminogen activator activity was enhanced after the 6-day treatment with EGF plus insulin and, particularly, with FGF plus insulin. Similarly, the response of the latter group to FSH was significantly higher than in any other group of cells. FGF was also able to stimulate cell multiplication and enhanced the FSH receptor number of Sertoli cells isolated from 15- and 26-day-old rats.
Conclusion: Thus, FGF is the most potent known mitogenic factor for cultured Sertoli cells, and it stimulates the phenotypic expression of these cells.

Keywords: Steroli Cells, Syrian Mice, FSH, Insulin, Plasminogen Activator

P-4: Prevention of Oxidative Stress Induced Changes in Semen Quality and Fertility Parameters of Male Wistar rat by Satureja Khuzestanica After Chronic Cyclophosphamide Exposure

Rezvanfar MA[1*], Sadrkhanlou RA[1], Ahmadi A[1], Rezvanfar A[2], Salehnia AN[2], Abdollahi M[2]

1. Laboratory of Embryology and History Department, Faculty of Veterinary Medicine, Urmia University, Iran
2. Laboratory of Toxicology, Department of Toxicology and Pharmacology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Iran
Email: amin2_dvm80@yahoo.com

Objective: The aim of this study was to investigate the possible protective role of Satureja khuzestanica essential oil (SKEO) a potent antioxidant on cyclophosphamide-induced spermiotoxicity using quantitative and biochemical approaches.
Materials and Methods: Adult male Wistar rats were divided into four treatment groups: (I) control, (II) 6 mg/kg CP once a day for 28 days by gavage, (III) 225 mg/kg SKEO once a day for 28 days by gavage and (IV) CP plus SKEO. Cauda epididymal sperm samples removed and analyzed for sperm concentration, motility, morphology and viability. The plasma samples were separated and kept at -80°C until analysis of oxidative stress markers (levels of lipid peroxidation and total antioxidant power). In addition, one testis kept frozen at -80°C until homogenized for further analyses of oxidative stress biomarkers. FRAP and TBARS assays were used to determine total antioxidant power and lipid peroxidation respectively. In addition mating studies were performed and different fertility parameters were assayed.
Results: Treatment of male rats with CP caused a significant decrease in the sperm count and motility, while dead and abnormal sperms increased as compared to control. Treatment with SKEO caused a significant improvement in semen quality and minimized the toxic effects of CP. The litter size and fecundity and fertility index of the male rats treated with CP was significantly lower than those of the controls. Administration of SKEO improved fecundity and fertility index and litter size significantly in CP-treated animals. Administration of SKEO reduced plasma and testis lipid peroxidation in comparison to control. Coadministration of CP and SKEO resulted in restoration of CP-increased lipid peroxidation in plasma and testis of CP-treated animals. Administration of SKEO increased plasma and testis total antioxidant power as compared to control. CP decreased plasma and testis total antioxidant power in comparison to control. Coadministration of CP and SKEO restored CP-induced reduction of total antioxidant power in plasma and in testis of CP-treated animals. Co-administration of SKEO with CP resulted in a significant recovery from semen disorders and oxidative stress in the testis and plasma.
Conclusion: The present results highly support the idea that reproductive toxicity of CP is mediated through oxidative stress and strong antioxidants like SKEO can protect the reproductive system form CP-induced damages that usually result in infertility.

Keywords: Cyclophosphamide, SKEO, Lipid Peroxidation, Antioxidants, Testicular Toxicity, Oxidative Stress, Semen, Fertility

P-5: The Effect of Ginseng as a Phytoestrogen on Busulfan-Induced Disorders in Seminal Parameters

Soleimani Rad SJ, Roshangar LR*

University, Embryology and Histology Department, Tabriz, Iran
Email: Lroshangar@yahoo.com

Objective: Destructive effect of busulfan as an anticancer drug on spermatogenesis well known. It is recently proposed that estrogen has a role on spermatogenesis.

The aim of present study in to evaluate the effect of ginseng as a phytoestrogen on busulfan-induced spermatogenesis disorder.
Materials and Methods: In the present study 40 balb-c mice were studied. The mice were kept under standard condition and divided in to 4 groups. In first group , mice received a single dose 20mg/kg busulfan intraperitoneally , dissolved in DMSO. Group B: in the second group the mice recived only DMSO. Group C: in the thread group after busulfan administration, the mice received 500mg/kg ginseng, orally, everyday for 40 days. Group D: fourth group considered as control group and received free food. the mice in all groups were sacrificed 40 days after busulfan administration and semen was collected from guada epididymies and placed in Hams F 10 medium add seminal parameters were evaluated and compared with control group.
Results: The results showed that the number of sperms were significantly decreased after busulfan injection (23.48±6.83 vs 33.23±6.39).while it was retrieved in those received ginseng. the other point is that in busulfan group the number of sperm between cases were vary variable. The number of sperm in ginseng receiving group increased comparison with busulfan receiving groups (32.60±4.37 vs 23.48±6.83). Regarding the motility sperms while the ginseng receiving groups had similar value the busulfan alone cased significant different. Percent of normal morphology in all groups were similar.
Conclusion: The results indicate that Ginseng as a phytoestrogen prevent spermatogenesis defect after busulfan injection. The results also show that busulfan do not increased sperm defect but affect stem cell proliferation and differentiation.

Keywords: Busulfan, Ginseng, Spermatogenesis

P-6: Evaluation of Antiandrogenic Potentials of Aqueous Extract of Chromolaena Odoratum (L.) K.R. Leaves in Male Rats

Yakubu MT*, Akanji MA, Oladiji AT

University of Ilorin, Biochemistry, Ilorin Nigeria
Email: tomuyak@yahoo.com

Objective: The antiandrogenic effect of oral administration of aqueous extract of Chromolaena odoratum leaves (250 and 500mg/kg body weight) for 14days in male albino rats was investigated.
Materials and Methods: Forty two white albino rats were randomly divided into three groups: A, B and C. Group A which served as the control received 1ml of distilled water (the vehicle) twice daily for 14days while groups B and C were treated the same way like the control except that the animals received 250 and 500mg/kg body weight of the plant extract respectively.
Results: Compared with the control, extract administration at 250 and 500mg/kg body weight revealed significant reduction ($p<0.05$) in testicular body weight ratio, acid phosphatase activities, protein, cholesterol, glycogen, sialic acid and testosterone concentrations with a significant increase ($p<0.05$) in lactate dehydrogenase and γ-glutamyl transferase activities. There was no significant change ($p>0.05$) in serum concentrations of follicle stimulating and luteinizing hormones. Histological examination revealed disruption in the arrangement of seminiferous tubules with no distinct basement membrane. These changes were accompanied by reduction in the number of spermatozoa.
Conclusion: All these results indicated that aqueous extract of Chromolaena odoratum leaves possess antiandrogenic property by interfering with steroidogenesis at the testicular level and this will adversely affect the functional capacity of the testes and the fertility of the animal.

Keywords: Chromolaena Odoratum Leaves, Aqueous Extract, Seminiferous Tubules, Antiandrogenic, Steroidogenesis

Embryology

P-7: Effects of Different Time of Exposure and Media with Varying Osmolarities on Viability and Motility of Najdi Bull Sperm Under 5 Degrees Centigrade

Afrough M[1*], Barati F[1], Papahn AA[2]

1. Fac. Vet. Med., Clinical Sciences, Ahvaz, Iran
2. Fac. Vet. Med., Basic Siences, Ahvaz, Iran
Email: m.afrough@gmail.com

Objective: Storage of semen in refregartor is one of possible method to preserve it. also for cryopreservation of sperm, it should pass a equilibration time in 5 derees of centigrade with basic cryopreservation medium (without cryoprotectant). aim of this study is to find the effects if time exposure (15 and 60 minute) and different osmolarities of media (0, 200, 300 and 400 mosm) have any detrimantatl of benificial effect on motility or viability of Najdi bull sperm.
Materials and Methods: Semen collected from 4 mature Najdi bull in station for support of Najdi cow, Khouzistan, Iran. primary media were made on the base of tryodes with osmolaities of 100, 300 and 500 mosm which were modified to 200, 300 and 400 mosm final osmolarities in combination of semen mixed. semen was cetrifuged in rich part of centrifuged semen was mixed in aequall proportion with deifferent media. 15 and 60 miute after production of mixute we analysed motility and vitality of sperm by conventional methods.
Results: Results have showed that time of exposure has not any compareble effect on vitality and motility of

bull sperm. After 15 minute motility of was significantly reduced in 200(12.5) vs 300 (28.62) ($p<0.05$) and there was not detrimental effect (p.0.05) on motility at 400 osmolaity medium (18.75) compare to 300 osmol.After 60 minute motility was significantly reduced in 200(2.82) vs 300 (34.25) ($p<0.05$) and there was not detrimental effect (p.0.05) on motility at 400 osmolaity medium (27.5) compare to 300 osmol. After 15 minute vitalty of was significantly reduced in 200(31.25) vs 300 (66.66) ($p<0.05$) and there was not detrimental effect ($p>0.05$) on viability at 400 osmolaity medium (45) compare to 300 osmol. After 60 minute vitalty of was significantly reduced in 200(2.82) vs 300 (34.25) ($p<0.05$) and there was not detrimental effect ($p>0.05$) on viability at 400 osmolaity medium (27.5) compare to 300 osmol.
Conclusion: Low temerature is good model for preserving the bull spermatozoa by lowering metabolic requirement and motility of sperm. In cryopreservation methods equlibration time can be reduced with no detrimental effect on sperm motility and viablity compare to long tome.

Keywords: Sperm, Bull, Cryopreservation, Osmolarity

P-8: Comparison of Survival Rate and Developmental Competence of Mouse MII Oocyte After Freezing by Slow Freezing and Vitrification

Agha-Rahimi A[1*], Kalantar SM[2], Azarnia M[1], Soleimani M[2], Mir Esmaili[2]

1. Tarbiat Moaalem University, Tehran, Iran
2. Research and Clinical Center for Infertility, Yazd, Iran

Objective: Oocyte freezing is the only method to preserve the reproductive capacity for woman at risk of losing it because of premature ovarian failure, pelvic diseases, and surgery, radiotherapy or chemotherapy treatments. But oocyte storage has faced technical difficulties compared with sperm or embryo cryopresevation because of specific structure of oocyte.The purpose of this study is investigate on the morphological survival rate and developmental competence of thawed oocytes after cryopreservation by slow freezing or vitrification.
Materials and Methods: Female mice super-ovulated with intraperitoneal injection of PMSG and HCG. Ovulated MII mouse oocytes were allocated to slow frozen and vitrified and control groups. Vitrification using ethylene glycol (EG) and dimethyl solfoxide (DMSO) and slow freezing using propanediol (PROH). After thawing the surviving MII oocytes in both cryopreserved and control group were inseminated for in vitro fertilization (IVF) and their developmental ability was compared.
Results: The survival rate of post thawed mature oocytes in vitrification group was 73.3% and in low freezing was 33.3%, ($P<0.01$). Then after IVF, The percentage of cleaved embryos in control, vetrification and slow freezing groups was 64%, 25% and 20%, respectively ($p>0.05$).
Conclusion: The result showed that survival rate after thawing in vetrification was greater than slow freezing, but the cleaved embryo rate did not differ significantly between the two different cryopreservation methods.

Keywords: Vitrification, Slow Freezing, Survival Rate, MII Oocyte, In Vitro Fertilization

P-9: The Effects of ELF-EMF on Fertility and Height of Mice Uterus and Fallopian Tube Epithelial Cells in Pre-Implantation Stage

Borhani N
Email: nsm_borhani@yahoo.com

Objective: The aim of the present study is to investigate the effects of extremely-low-frequency electromagnetic field on fertility and height of mice uterus and fallopian tube epithelial cells in pre-implantation stage.
Materials and Methods: Eighty female NMRI mice were randomly divided into 2 groups: control group was not exposed to EMF and treatment group was exposed to 4 hours per day, 6 days a week for 2 weeks to 50 Hz & 0.5 mT EMF. Female mice in all groups were superovoulated and mated over night. Next morning females with a vaginal plug were identified as pregnant mice; at the time of implantation pregnant mice were anaesthetized and blastocysts were subsequently obtained from these mice by flushing the uterus horns. The samples of Uterus horns and fallopian tubes in all groups were taken and were processed for light microscopic studies.
Results: Results showed that the mean number of pregnant mice decreased in EMF group (50%) compared to the control group (67.5%) but the difference between them was not significant. The mean number of fetuses per pregnancy was (9±4.8) in control group and (5.5±5.7) in treatment group and statistical analysis were showed significant decrease between mean of 2 groups ($p<0.03$). The analysis showed that height of uterus epithelial cells is increased in treatment group but this difference wasn't significant (46.09±6.08, 44.71±5.41). In addition, results show significantly increase in height of fallopian tube epithelial cells in EMF group compared to control group (52.97±5.96, 45.27±3.50 & $p<0.000$).
Conclusion: The results show that ELF-EMF decreased the pregnancy rate and this decrease associated with increase of fallopian tube epithelial cells height.

Keywords: ELF-EMF, Pre-Implantation, Epithelial Cell, Uterus, Fallopian Tube, Mouse

P-10: The Effect of Super Ovulation on Quality of Mouse Blastocyst

Darabi M*, Bayat P, Sheikholeslami B

Anatomy Departemtn, Arak University of Medical Sciences, Arak, Iran
Email: m_darabi36@yahoo.com

Objective: At present the super ovulation is routinely used in order to increase the number of oocytes and embryos by injection of PMSG & hCG. Although there are many successes in advertisement of these drugs, the failure of fertilization & decreasing the cleavage rate & preimplantation & implantation impairment are reported. In order to overcome these problems, we should survey the effects of these drugs on fertilization, growth and development of embryos.
Materials and Methods: The NMARI female mousses were superovulated with IP injection of PMSG & hCG. The pregnant mouse were killed and their oviducts flushed with RPMI 1640 medium and 2-cells, 3-4 cells, 5-8 cells, morolla and blastocysts embryos were collected and studied at certain times with stereomicroscopy. The blastocysts were stained by double staining methods (Hoschest) in order to study and compare the quality of embryos by fluorescent microscope. The number of alive embryos produced by IVF following superovulation, were studied to determine the fertilization rate.
Results: There was a significantly difference between mean of superovulation and control groups (p=0.05). The case and control groups have significant difference for number of 1-cell, 2-cells, 3-4 cells, 5-8 cells, morulla and blastocysts embryos (p=0.05). The qualities of embryos haven't significant difference between two groups by counting the ICM & TE cells. To analyze the data of this study we compared the mean number of embryos by paired t-test.
Conclusion: Our results showed that also the embryo fertilization and development were affected by superovulation (by PMSG & hCG), but when we used the suitable dose, the blastocyst quality weren't affected and they will be probably impact.

P-11: Zeta Method: A Novel Sperm Selection Method for ICSI with Normal Chromatin Structure

Kheirollahi Kh.M*, Nasr Esfahani MH

Andrology Department, Cell Sciences Research Center, Royan Institute(Isfahan Compus), ACECR, Tehran, Iran
Email: mkheirollahi_biologist@yahoo.com

Objective: Sperm selection for ICSI only depends on morphology and motility, but these parameters may not the relevant to chromatin integrity. So sperm selection procedure based on sperm functional characterized has been suggested. One of these procedures is Zeta method. In this procedure sperm is selected based on presence of negative charge on sperm membrane. Thus, the aim of this study was the comparison between pure gradient and Zeta method, to select spermatozoa with normal chromatin. In addition, evaluation of fertilization and pregnancy rate between two groups was carried out.
Materials and Methods: Semen samples collected from infertile couples referring to Isfahan Fertility and Infertility center for ICSI treatment. A portion of semen was used for routine semen analysis and the remainder was divided to three groups, control, Zeta method and pure gradient procedure for ICSI. Then, for all groups, we evaluated protamine deficiency using Chromomycin A3 (CMA3) and sperm chromatin dispersion (SCD) test for DNA fragmentation.
Results: The percentage of DNA fragmentation and protamine deficiency has reduced significantly in the Zeta method compared to control group ($p<0.05$). Furthermore, above parameters were significantly reduced in the pure gradient method compared to control group ($p<0.05$). In addition, pregnancy rate in patients who were candidate for ICSI based on Zeta selection were significantly higher than patients who only underwent conventional ICSI (pure gradient group).
Conclusion: Using the zeta method for selecting normal spermatozoa with intact DNA and protamine content is an appropriate method for ICSI treatment.

Keywords: Zeta Method, DNA Integrity, Protamine Deficiency, ICSI

P-12: Vitrification of Cumulus-Germinal Vesicle Break Down (GVBD) Oocyte Complexs and the Assessment of Cumulus Viability, In Vitro Maturation (IVM) and In Vitro Fertilization (IVF) of Them

Khosravi Farsani S[1*], Abbasi M[1], Mahmoudi R[2]

1. Embryology, Anatomy, Tehran, Iran
2. Embryology, Anatomy, Yasuj, Iran
Email: s_farsani@yahoo.com

Objective: Cryopreservation is still an open challenge in most mammalian species. According to literatures, the oocytes have distinct properties in each species and in each nuclear maturity. Cumulus cells are thought to protect the oocyte against cell damage during cryopreservation. The damage occurring to the cumulus cells surrounding the oocytes could affect the success of immature oocyte cryopreservation. The aim of this study was to investigate the effects of vitrification on cumulus preservation and post-thaw oocytes maturation and fertilization.
Materials and Methods: COCs were cultured for 3 hr in TCM199 medium in a humidified atmosphere of 5% CO2 in air at 37°C. After getting to GVBD, oocytes were randomly allocated into three groups. (1) Control (underwent 21 hr maturation without vitrification), (2) Exposed to single-step vitrification (contained of EG 30%+0.5M sucrose), (3) Exposed to step-wise vitrification (2%, 5%, 10%, 30%EG +0.5M sucrose). Thawing was carried out in four steps using sucrose solution in a holding medium. After thawing oocytes underwent additional 21 hr maturation. MII oocytes were subjected to in vitro fertilization. Oocytes viability, maturation to MII and 2-cell stage was analyzed using inverted microscope. Additionally viability was assessed by stain-

ing of Ho /PI. Viable cumulus cells were counted by Neubauer counting.
Results: All non-vitrified oocytes were viable after 24 hr; however, viability and maturation rate of vitrified samples in single-step group was significantly lower than that of the step-wise and control Groups ($p<0.05$). Cumulus cells viability in step-wise group was lower, not significantly, than that in control group. In step-wise vitrification distribution of cumulus cells did not occur after dissolution. In step-wise group, cleavage (2-cell) rate was significantly higher than that of the single-step group ($p<0.05$). However, Development to 2-cell stage from the vitrified oocytes was much lower than that of the control group ($p<0.05$).
Conclusion: Our results suggest that step-wise vitrification of GVBD oocytes was better in rate of survival, in vitro maturation (IVM) and in vitro fertilization (IVF) of oocytes. Also, step wise vitrification was superior in viability and preservation of cumulus cells.

Keywords: Vitrification, Viability, In Vitro Maturation, In Vitro Fertilization, GVBD

P-13: The Effect of Ovarian Tissue Vitrification on Expression of p53 in Cultured Ovarian Follicle

Mazoochi T[1*], Salehnia M[2], Rezazadeh Valojerdi M[2]

1. Histology and Pathology Departemtn, Kashan University of Medical Sciences, Kashan, Iran
2. Anatomy Departemtn, Tarbiat Modares University, Tehran, Iran
Email: mazoochi45@ yahoo.com

Objective: Vitrification is a simple and ultra rapid technique for the conservation of fertility. This study was carried out to evaluate the effect of mouse ovarian tissue vitrification on the expression of p53 in ovarian follicle during culturing.
Materials and Methods: This experimental study was carried out 12-14 day- old female mice (NMRI). Ovaries were vitrifies with a solution containing ethylene glycol. After fast warming, preantral follicles mechanically isolated from vitrified and non vitrified ovaries and individually cultured in α-MEM (Gibco,UK) supplemented with 5% FBS, 100 mIU /ml rFSH, 1% ITS and 20 ng/ml mrEGF nonspherically for 10 days. Expression of apoptotic genes p53 were evaluated in isolated in-vitro maturated follicles from vitrified-warmed ovaries and compared with nonvitrified samples. Total RNA extracted from all above mentioned groups and reverse transcripted by oligo dt primer and M-MLV enzyme. cDNA product amplified by specific primer pairs for p53 and β2m (as an internal control) during PCR. The semiquantitative expression of p53 mRNA in every stages of follicle was compared using post-hoc LSD test.
Results: Data showed expression of p53 in different stages of follicles in two groups of study. The relative abundance of p53 mRNA to the β2m was similar in all groups. p53 mRNA was strongly expressed in preantral follicles and was lower in antral follicle in vitrified and nonvitrified groups. Relative level of p53 did not change significantly in three stages of follicles in vitrified and nonvitrified samples.
Conclusion: We concluded that ovarian vitrification using ethylene glycol has no significant impact on expression of p53 in different stages of follicles during culturing.

Keywords: Vitrification, Ovary, p53, RT-PCR, Follicular Culture

P-14: Rose Water Negative Effect on Number of Implantation Sites in Mice Uterus

Mehrabi-Nasab E[1], Khazaei M[2*]

1. Medical Faculty of Kermanshah, Reproduction Research Center, KermanshahIran
2. Dentistry Faculty, Student Research Center, Isfahan, Iran
Email: mkhazaei1345@yahoo.com

Objective: Pure rose water was used in nutritional and chemical manufactures and traditional medicine. It consumed in dried skin and irregular menstrual period, also it can affect fertility. The aim of present study is to determine effect of pure rose water on number of implantation site in mouse uterus.
Materials and Methods: Female virgin albino mice with 20-25 gr weight were divided in 3 groups (n=10): one control and two cases, 10 and 20 mg/kg of rose water. After overnight contact between female and male mice and finding vaginal plug, control group received normal saline and case groups received, 10 and 20 mg/kg/day of rose water in same volume for five days by intraperitoneal injection. All animal were killed in 7th days of pregnancy and their uterus dissected out. Implantation sites were count by stereomicroscope. Data were analyzed by one way ANOVA and $P< 0.05$ considered significant.
Results: There was significant difference between control and case groups (10 and 20 mg/kg/day). Intraperitoneal injection of rose water decreased implantation sites in mouse uterus. There was no significant difference between cases groups.
Conclusion: Pure rose water exerts negative effect on mouse fertility by decreasing number of implantation site.

Keywords: Rose Water, Implantation Site, Mice, Fertility

P-15: Effects of Cysteamine on Rate of In Vitro Maturation of Oocytes (IVM) in Two Media

Noori Mugahi SMH.

Histology Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: nooromoo@sina.tums.ac.ir

Objective: Rate of in vitro maturation of oocytes is one of the challenge of assisted reproductive techniques. In this study we investigated effects of supplementation of cysteamine on rate of in vitro maturation of oocytes in two different media. Germinal vesicle oocytes were collected from mouse ovary.
Materials and Methods: Oocytes were cultured in two media (TCM199 and MEME) with 0, 50, 100, 200, 500 μM/ml cysteamine. Number of germinal vesicle breakdown (GVBD) and metaphase II (MII) oocytes were recorded. The results showed that, rate of IVM in 100 μM/ml cysteamine was high significantly compare to control ($p<0.05$).
Results: Evaluation of two media in this study showed that TCM199 improved rate of IVM and oocyte maturation better than MEME, however this difference was not significant.
Conclusion: These findings indicated that TCM199 in compare to MEME was better in rate of in vitro maturation of oocyte .

Keywords: In vitro maturation of oocyte, Cysteamine, Glutathione, Mouse

P-16: Distribution of Integrins in the Mouse Endometrium During the Oestrous Cycle

Peyghambari F[1*], Salehnia M[1], Forouzandeh Moghadam M[2], Rezazdeh Valujerdi M[1], Hajizadeh E[3]

1. Department of Anatomy, Tarbiat Modares University, Tehran, Iran
2. Department of Biotechnology, Tarbiat Modares University, Tehran, Iran
3. Department of Biostatistics, Tarbiat Modares University, Tehran, Iran
E-mail: salehnim@modares.ac.ir, mogdeh@dr.com

Objective: Integrins have recently been proposed as having a major role in endometrial receptivity. The aim of this study was investigation of different patterns of integrin expression during the normal endometrial oestrous cycle.
Materials and Methods: Stages of estrous cycle were determined in mice by analysis of vaginal smears then they were sacrificed by cervical dislocation and the tissues were obtained from the middle 1/3 part of their uterine horns immediately and processed for studies immunostaning.
Results: Integrins was maximally expressed in the metestrus phase in endometrum. Integrin beta 3, alpha 4 and beta 1 were detected in luminal and glandular epithelial but alpha v was detected only in glandular epithelial. Integrins of alpha v and beta 3 in the stroma were seen too.
Conclusion: Expression of integrins in metestrus phase of oestrous cycle in mouse around the time of the implantation window suggest a role for these proteins in endometrial function and implantation.

Keywords: Endometrium, Mouse, Integrin, Immuno-Histochemistry, Implantation

P-17: In Vitro Follicular Development of Cryopreserved Ovarian Tissues Isolated from Syrian Mouse

Rezaei-Zarchi S[1*], Javed A[2]

1. Payam-e-Noor University, Biology Department, Yazd, Iran
2. University of Agriculture, Biochemistry Departemtn, Faisalabad, Pakistan
Email: srezaei@ibb.ut.ac.ir

Objective: The purpose of this study was to define a culture system with some technical modification for preantral follicles isolated from frozen/thawed ovarian tissue and to confirm cell injury.
Materials and Methods: Ovaries obtained from three-week-old female mice were cryo-preserved by the rapid freezing method. Preantral follicles isolated from frozen/thawed ovarian tissues were cultured for 12–16 days. The follicles were then stimulated with human chorionic gonadotropin (hCG). In vitro fertilization was performed on the released cumulus-oocyte complexes (COCs).
Results: Preantral follicle viability was assessed by supravital staining using Hoechst 33258. By using this stain, the cell death was found in part of the granulosa cells of a follicle obtained from frozen/thawed ovarian tissue. On the 14th and 16th days of culture, the diameters of follicles isolated from frozen/thawed ovaries were larger than on the 12th day of culture. The released COCs were fertilized and developed to the blastocyst stage in 15.8% (12/76) of the oocytes taken from the fresh group, and in 0% (0/73), 2.9% (2/69) and 19.1% (22/115) of the oocytes taken from the frozen/thawed group that had been cultured for 12, 14 and 16 days respectively. The preantral follicles isolated from frozen/thawed mouse ovarian tissues developed slowly compared with the freshly prepared preantral follicles. During prolonged culture from 12 to 16 days, these follicles obtained the potential to fertilize and develop to the blastocyst stage.
Conclusion: In conclusion, we have demonstrated that cryo-preservation of mouse ovarian tissues, by rapid freezing, is successful in allowing the oocytes to maintain their ability to undergo meiosis and preimplantation development. The freeze/thaw process may have some effects on growth suppression of the oocytes themselves and of granulose cells. Our study using mouse ovaries may provide the basis of clinical applications to human folliculogenesis for female gamete conservation.

Keywords: Follicles, Cryo-Preservation, Oocytes, Development, Freeze/Thaw, Gamete

P-18: Effect of Serum Starvation on Proliferation, Apoptosis and Cell Cycle Stage in Granulosa Cell

Sadeghian Nodushan F[1*], Eimani H[1], Eftekhari Yazdi P[2], Sepehri H[3]

1. Embryology Department, Cell Sciences Research Center, Royan Institute, ACECR, Tehran, Iran
3. Tehran University, Biology Department, Tehran, Iran
Email: fsadeghian@gmail.com

Objective: Many factors affect the efficiency of NT. One important factor is the cell cycle phase of the donor cells at NT. Serum starvation and prolonged culture to confluency are commonly used methods to synchronize cells in the G0 or G0/G1 phase of the cell cycle, respectively. However, experiments differ in the duration of serum deprivation and concentration of serum. The efficiency of serum withdrawal in controlling cell cycle and the effect of long-term starvation on cell requires further investigation. So, in this study, Serum starvation effect on granulosa cells which are frequently and successfully used as donor cells in cloning experiments has been investigated.
Materials and Methods: Granulosa cells were aspirated from ovarian follicles and plated in a DMEM medium containing 15% FBS. Upon 70-80% confluency, the medium of the primary-cultured as well as the passaged-5 cells were replaced with the medium containing either 0.5% FBS for 24, 48 and 72 h. When cells became confluent, they harvested by treatment with 0.05 % Trypsin.-EDTA, the reaction was terminated by DMEM and the suspension was passaged (5th passage). cells were examined in terms of their cell cycle stage using flow cytometry. Moreover, the cultures were investigated with respect to their apoptotic as well as the proliferating cell contents using Brdu labeling and tunnel staining.
Results: At primary as well as passaged-5 cultures subjected to serum starvation for 24 h, the frequency of G0/G1, proliferating as well as apoptotic cells were similar to those from control group. At culture with 48 and 72h serum starvation, the percentage of G0/G1 cells tended to significantly be increased to 83% and 85% at primary culture and 89% and 90% at passage-5 culture respectively. The percentages of apoptotic cells in cultures with either serum starvation for 24 and 48 h were not increased compared to those from control cultures. According to our results, 72 h after serum starvation, frequency of the apoptotic cells appeared significantly to be increased.
Conclusion: Serum starvation for 48 h or 72h could increase the amount of Go/G1 cells but Serum starvation for 3 days significantly promoted apoptosis in granulosa cells.

Keywords: Synchronization, Confluency, Serum Starvation, Granulose Cells

P-19: Effects of Increased Ambient Temperature During IVM and/or IVF on the In Vitro Development of Bovine Zygotes

Sugiyama S[1], Kafi M[2*]

1. Queesland University of Technology, QLD, Australia
2. Shiraz University, Shiraz, Iran
Email: s.sugiyama@qut.edu.au

Objective: This study was designed to examine the effects of heat treatment (HT) during in vitro maturation (IVM) and/or during in vitro fertilization (IVF).
Materials and Methods: Immature cumulus oocyte complexes (COCs) were collected from abattoir-derived ovaries and used in this in vitro study. One 24 h cycle of HT entailed a series of 0.5°C incubator temperature increase from 39°C to 39.5°C for 2 hrs, to 40°C for 2 h, to 40.5°C for 4 h, 41°C for 4 h, 40.5°C for 6 h and 40°C for 6 h. Experiment I studied the effects of one cycle of HT during IVF on the rate of cleavage of in vitro matured presumptive zygotes. Experiment II repeated the HT of experiment I but preceded it with a cycle of HT during IVM. Experiment III examined the rates of embryonic development to ≥8 cell stage (after 72 h IVC) and to morula or blastocyst (M/B) stage (after 144 h IVC) following HT of the oocyte groups during the preceding IVM or IVF.
Results: Total cleavage rate in the HT group (37.8%) was lower than that of the control group (54.6%, $p<0.05$) in experiment I. The total cleavage rates for control and heat treatment heat treatment groups were 75.5% and 37.9% respectively with a significant difference of $p<0.001$ identified in experiment II. Rates of development to ≥8 cell stage (at 72 h IVC) and to M/B stage (after 144 h IVC) for the control group were 27.5% and 35.8% in experiment III. Those of IVM-only HT and IVF-only HT group were 13.8% and 14.6% and 8.6% and 14.3% respectively. Both groups of heat treated embryos developed at significantly lower rates ($p< 0.05$) than did the control group.
Conclusion: The results of this study suggest that hyperthermia during oocyte maturation and/or fertilization adversely affects oocyte maturation and fertilization rates and retards further embryonic development.

Keywords: Bovine Zygotes, Heat Treatment, IVM, IVF

Epidemiology and Ethics

P-20: Contribution of Religion and Spirituality in Adjusting Infertile Women's Marital Relationships

Latifnejad R[1*], Allan HT[2], Smith PA[2]

1. Mashhad University of Medical Science, Mashhad, Iran
2. Division of Health & Social Care, Faculty of Health & Medical Sciences, Universityof Surrey, United Kingdom
Email: r_latifnejad@hotmail.com

Objective: Despite growing body of literature regarding marital adjustment of infertile women, there is no study to address the role of religious and spiritual beliefs in adjusting marital relationships in infertile women. Considering the significance of marital cohesive-

ness and commitment in the long and stressful journey of infertile women, this study was designed to explore the experiences related to infertility in a marital relationship context and to illuminate how marital relationships is experienced by infertile women who affiliated to different religious faiths.
Materials and Methods: For this study a group of 30 infertile women affiliated to different denominations of Christianity (10 Protestants, 6 Catholics, 2 Orthodoxies) and Islam (6 Shiites and 6 Sunnis) were interviewed. The design was a feminist grounded theory study including semi structured in-depth interviews. Data were collected in one Iranian and two UK fertility clinics through theoretical sampling and analyzed using Nvivo software, Version 2. consistent with Strauss & Corbin's mode of grounded theory.
Results: Religious infertile women using a religious/ spiritual meaning-making framework tried to perceive their marital life as something granted by God which could be accepted peacefully. They tried by establishing a divine relationships and going through the following phases adjust their marital relationships: being optimistic and positive, having supportive relationships, being grateful and appreciated for their marital life, offering spiritual sympathy and adopting religious role models. These strategies aided infertile women and their husbands to be more understanding, sympathetic and gentle to each other and maintain a family cohesion.
Conclusion: I argue that awareness of health professionals of the potential ways in which religion and spirituality assist infertile women to deal with their marital issues could be important. This knowledge will help them to support emotional wholeness and integrity of infertile women, offering religious and spiritual coping strategies which can help adjusting their marital relationships.

Keywords: Infertile Women, Religion, Spirituality, Marital Relationships, Feminist Grounded Theory

P-21: Life Styles for Reprocuctive Health Promotion Among Young College Girls

Rao AS[1*], Rao RM[2]

1. PVP Siddhartha Inst of Tech, Health Edn & Sports Sciences,Vijayawadaindia
2. PVP Siddharths Inst of Tech, Admin amd Nationalsocia Service,Vijawadaindia
Email: sudhakararao_anche@yahoo.co.in

Objective: The specific objectives of the life styles are to accommodating learning style preferences in achieving reproductive health, recognizing and understanding the factors that contribute to the positive aspects of reproductive health among young college girls. Life styles to modify risk behaviors and develop new improved behaviors through stress control, meditation, fitness, nutrition and relaxation techniques management. Encourage the young girls to control life style behaviors motivate to assume an active role in the maintenance and improvement of health condition that promote low risk behaviors.
Materials and Methods: Adopting qualitative research design on young college girls through selected life styles to accommodate learning style preferences as communication strategy to understand adolescent age physical and emotional changes and to develop positive reproductive health behaviors – caring reproductive health, skills for correct contraceptive measures, using resistance skills to avoid adolescent pregnancy, and unprotected sex, pre marital sex consequences and choose behaviors to reduce the risks of STD/STI. Life styles for fitness, nutrition meditation and psychological health parameters stress management and relaxation techniques also implemented as study interventions for better physical, mental and spiritual health and low risk behavior achievements. The selected parameters are evaluated for finding the results comparing pre and post interventions.
Results: The findings of the life styles learning preferences reported significantly on young college girls' reproductive health. The evaluated reproductive health perceptions, pre and post interventions reproductive health care, adolescent pregnancy, unprotected and premarital sex, contraceptive skills and STI/STD risks have very positive results. The implemented lifestyles on fitness, nutrition, physical, mental and spiritual health have success in the promotion of selected parameters.
Conclusions: The study confirms the positive effects on life styles approaches including reproductive health, fitness, nutrition, physical, mental and spiritual health through adolescent age physical and emotional changes control among young girls.

Keywords: Modified Risk Behaviour Positive Reproductive Health Resistance Skills Pregnancy and Premarital Sex Psychological Health Life Style Preferences

Female Infertility

P-22: Technical Problems of HSG

Ahmadi F, Zafarani F*, Vosough Dizaj A

Diagnostic Imaging Division, Endocrinology and Female Infertility Department, Royan Institite, ACECR, Tehran, Iran

Investigation of the intrauterine cavity and tubal patency is indicated for many clinical conditions in gynecology. Despite the varied diagnostic options such as hysteroscopy and laparoscopy, hysterosalpingography is still an important and complementary examination in the early evaluation of infertility.
The technique of HSG is quite simple, less invasive, more convenient, and provide reliable information about

the uterine cavity, tubal patency, lesions, congenital anomalies and different types of intrauterine defects at less cost. Other than diagnostic, it can be therapeutic at time.
Utilization of proper procedure can provide valuable diagnostic information and limit technical errors.
A variety of technical problems may occur during HSG. These may relate to instrumental malfunction, anatomic abnormalities, artifacts or functional disturbances, and patient discomfort causing termination of the examination.
The technical difficulties of HSG with an emphasis on the ways of facing problems are addressed in this presentation.

P-23: Effects of Exogenous Ghrelin Administration on Hormonal Profile in Female Sannan Goats

Farifteh F[1*], Khazali H[1], Emami M[2]

1. College of biological Sciences, University of Shahid Beheshty, Tehran, Iran
2. Center of Agricultural Institute of Yazd, Iran
E-mails: f.farifteh@yahoo.com

Ghrelin is a 28-amino acid peptide which is secreted from brain and gastrointestinal system and primarily involved in the control of food intake and growth hormone secretion. Based on its neuron distributions in hypothalamus, ghrelin coexists with many other neurons and displays multiple endocrine and non-endocrine actions. Therefore, ghrelin controls different physiological actions on many different tracts; it participates in the modulation of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid axis functions. The present experiments were carried out to analyze the potential involvement of ghrelin in the control of gonadotropin and thyroid hormones secretion.
Therefore the goal of this study was to determine whether ghrelin effects on the mean plasma concentration of Triiodothyronine (T3), Tetraiodothyronine (T4), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in the female Sannan goats.
Forty female Sannan goats were randomly divided into two groups. Animals in each group received daily injections of either 1 or 2 mg ghrelin/Kg BW (body weight) into jugular vein everyday for ten days. Blood samples were collected every 30 minutes after injection of ghrelin for two hours from three days before first injection till three days after last injection; Samples were assayed for plasma FSH, LH, T3 and T4 concentration by Radioimmuno assay (RIA) technique.
Injection of 1 and 2 mg ghrelin/Kg BW decreased the mean plasma concentration of LH throughout the injection period among all animals in two groups. The result of these experiments indicated that ghrelin significantly decreased mean plasma concentration of LH in the female Sannan goat ($p<0.01$). But, it has no significant effect on the mean plasma concentration of FSH, T3 and T4.
In the present study it was concluded that ghrelin has no effect in the secretion of FSH, T3, and T4, but has an inhibitory effect on LH.

Keywords: Ghrelin; Follicle Stimulating Hormone; Luteinizing Hormone; Triiodothyronine; Tetraiodothyronine; Sannan Goat

P-24: Knowledge and Attitudes of Nurses and Midwifes About Infertility in Tabriz

Ghatreh Samani Z

Tabriz Azad University, Nursery, TabrizIran
Email: zohreh_s2003@yahoo.com

Objective: The first line for guiding the infertile couples are always General practitioners, nurses and midwives and in some extend if available, gynecologists. It is very important to give the right information preventing any harm to the infertile couples. To give the right information needs high knowledge between them. This study was designed to evaluate the knowledge and attitude of nurses and midwives as the first line of patient guidance about infertility.
Materials and Methods: A questionnaire was designed to assess primary knowledge about the infertility and the new treatments of it including in vitro fertilization and microinjection. The sample contained nurses and midwives from general hospitals in Tabriz including 29 Bahman, Sina, Ali Nasab hospitals. The special gynecology and obstetrics hospital was excluded to prevent any bias. A sum of 54 nurses and 38 midwifes accepted to participate in our survey. Midwives were all female and 34% of the nurses were male. There were both open and colsed questions in the questionnair.
Results: In definition of infertility 62% of midwives and 34% of nurses had correct answers. In female and male causes of infertility 68% of midwives and 38% of the nurses had correct answers. In explanation of IVF 78% of midwives and 41% of the nurses were correct but in ICSI explanation only 47% of midwives and 28% of the nurses were correct. Nobody could explain the preimplantation genetic diagnosis and laser hatching, but 59% of midwives and 47% of the nurses could give a definition of cryopreservation of the embryo or gametes.
Conclusion: Nurses and midwives as the first line of consultation with the infertile couples have less information about the infertility and maybe misguide the patients. It was clearly shown that midwives' knowledge was better that probably because of their education. There was no significant difference between male and female nurses. Newer procedure like PGD and laser hatching needs to be taught to the clinical staff to increase their knowledge in these fields.

Keywords: Knowledge, Attitude, Nurses, Midwives, Infertility

P-25: Tamoxifen Alters Hormonal Levels in Adult

Rat and Folliculogenesis in Fetus

Hamidi M[1*], Malekinejad H[2], Ahmadi A[3], Sadrkhanlou R[1]

1. Veterinary Faculty, Histology and Embryology Department, Urmia University, Urmia, Iran
2. Veterinary Feculty, Pharmacology and Toxicology Department, Urmia University, Urmia, Iran
Email: hamidivet@gmail.com

Objective: Tamoxifen (TAM) is a synthetic, non-steroidal, anti-estrogenic/competitive antagonist, which is widely used for treatment of early and metastatic breast cancer (1). It's anti-estrogenic effect appears to be related to its ability to reduce estrogen receptor levels or to inhibit the binding of oestradiol (E2) to the estrogen receptors (ER). Although TAM acts primarily as an anti-estrogenic, it also exerts a mild estrogenic effect (2). We conducted an experimental study to evaluate the effect of TAM toxicity on the folliculogenesis in rat's fetus. Moreover, hormonal situ also were measured.
Materials and Methods: Tamoxifen (TAM) is a synthetic, non-steroidal, anti-estrogenic/competitive antagonist, which is widely used for treatment of early and metastatic breast cancer (1). It's anti-estrogenic effect appears to be related to its ability to reduce estrogen receptor levels or to inhibit the binding of oestradiol (E2) to the estrogen receptors (ER). Although TAM acts primarily as an anti-estrogenic, it also exerts a mild estrogenic effect (2). We conducted an experimental study to evaluate the effect of TAM toxicity on the folliculogenesis in rat's fetus. Moreover, hormonal situ also were measured.
Results: The histological examinations of fetus's ovary and biochemical data showed the significant changes in rats treated by TAM with the absence of folliculogenesis and an increase in E2 level which accopmanined with sharp decrease of FSH level in comparison to their respective control group.
Conclusion: In conclusion, this obtained data may suggest a mechanistic pathway of TAM action which takes place via changing of hormonal situ on one hand and direct effect on folliculogenesis. Moreover, as this agent is considered as group D in pregnancy period, thus more attention should be put on prescribing of this medicine.

Keywords: Tamoxifen, Ovary, Folliculogenesis

P-26: Effect of Ascorbic Acid and α-tocopherol on the in vitro growth and maturation of Syrian mice follicles and enclosed oocytes in the presence and absence of FSH

Javed A[1, 4*], Rezaei-Zarchi S[2], Javeed Ghani M[3], Jamil A[4*], Kalantar SM[1, 5]

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Biology, Payam-e-Noor University, Yazd, Iran
3. Department of Bioinformatics, Government College University, Faisalabad, Pakistan
4. Molecular Biochemistry Lab; Department of biochemistry, University of Agriculture, Faisalabad, Pakistan
5. Yazd Medical Biotechnology and Genetic Engineering Incubator, Yazd, Iran
Email: libraforever_2006@yahoo.com

Objectives: The objective of the present study was to demonstrate the effect of ascorbic acid and α-tocopherol on the preantral follicles and enclosed oocytes in the presence and absence of optimal amounts of FSH.
Materials and Methods: Preantral follicles were isolated from the ovaries of 6 week-old female mice and cultured in TCM-199 medium. Firstly, the optimization of FSH concentration was done for the in vitro culture. Different concentrations (10, 50, 100, 200, 300 and 400 nmol/ml) of the test vitamins were then added to the culture medium in the presence and absence of 100 mIU/ml FSH during separate experiments. Follicles were incubated for 6 days at 37 °C, 92% humidity and 5% CO2 in air.
Results: FSH concentration of 100 mIU/ml showed increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates ($P<0.0001$). However, 300 nmol/ml ascorbic acid and α-tocopherol showed a significant increase in the percentage of surviving follicles as compared to control (59 and 55%, respectively; $p<0.001$), while follicular diameter, GVBD and oocyte maturation rates were unaffected by all the concentrations used. In the presence of 100 mIU/ml FSH, 300 nmol/ml ascorbic acid and α-tocopherol, the diameter did not show any significant increase as compared to the FSH-treated cultures (190 μm), while the survival rate was recorded to be 95%, which was significantly more than that seen for FSH-treated cultures (91%; $p<0.05$) and the control experiment ($p<0.0001$). Significantly increased GVBD (89%) and oocyte maturation (71%) percentages were also seen ($p<0.0001$) during the fully supplemented medium.
Conclusion: The present study reported, for the first time ever, that FSH, α-tocopherol and ascorbic acid increase the survival rate of the follicles and enclosed oocytes, isolated from immature Syrian mice.

Keywords: Follicle Stimulating Hormone, Ascorbic Acid, Preantral Follicles, α-Tocopherol, Oocyte Maturation, GVBD

P-27: Foenicullum Vulgare Alcoholic Extract Promotes Folliculogenesis in Female Albino Mice

Khazaei M*, Montaseri A, Khazaei MR

Reproduction Research Center, University of Medical Science, Kermanshah, Iran
Email: mkhazaei1345@yahoo.com

Objective: The aim of the present study is to investigate the effects of Foenicullum Vulgare (FVE) on the folliculogenesis in female albino mice. FVE is used in traditional medicine for its antiseptic, palliative, anti-inflammatory and estrogenic effects.
Materials and Methods: Twenty female albino mice divided into 4 groups. Groups 1 and 2(Cases) received FVE alcoholic extract at dose of 100 and 200 mg/kg/day for five days, group 3 and 4 were administered ethanol and normal saline respectively as same dose as cases. Animals in all groups sacrificed on the 6th day of study and their ovaries dissected out and prepared for histological examinations.
Results: There was a significant increase in the number of graffian, antral and multilaminar follicles in the present of 100 and 200mg/kg doses of FVE extract, but there was no significant difference in follicle numbers among the FVE doses. Also there was no significant difference in the number of unilaminar primary follicles between all case and control groups
Conclusion: FVE alcoholic extract had folliculogenesis effect on mouse ovary and produce significant increase in the number of follicles.

Keywords: Foeniculum Vulgare, Folliculogenesis, Alcoholic Extract, Ovary

P-28: How Do Religious and Non-Religious Infertile Women Diverge in their Perspectives and Practices Towards ART?

Latifnegad R[1*], Allsn H[T2], Smith PA[2]

1. Mashhad University of Medical Science, Mashhad, Iran
2. Division of Health & Social Care, Faculty of Health & Medical Sciences, Universityof Surrey, United Kingdom
Email: r-lifnejad@hotmail.com

Objective: Literature has demonstrated that religions have similarly responded to ART, but little attention has been given to the perspectives of followers of diverse religions and also non-religious infertile women towards ART. This study explored the perspectives and practices of religious and non-religious infertile women in relation to ART.
Materials and Methods: In this study using a feminist grounded theory approach 30 infertile women affiliated to different denominations of Christianity (10 Protestants, 6 Catholics, 2 Orthodoxies) and Islam (6 Shiites and 6 Sunnis) and also 7 infertile women with no formal religion were interviewed. Volunteer participants were purposively recruited in one Iranian and two UK fertility clinics and the sample size was determined by theoretical sampling and data saturation. Data were collected through semi structured in-depth interviews, post interview notes, research diaries and a quantitative tool entitled Religious Spirituality Assessment Inventory. Data analysis was carried out using Strauss and Corbin's mode of grounded theory by means of Nvivo software, Version 2.
Results: All participants either religious or non-religious explored medical expertise, particularly in relation to ART to resolve their fertility problem. However, religious participants concurrently sought religious prohibitions and authorizations to manage undertaking ART. In terms of decision-making to accomplish IVF, non-religious participants and also the mainstream of Muslims and Christians deemed IVF as a kind of advanced technology of reproduction with no religious prohibition, although few Protestants and Catholics viewed IVF as a kind of meddling with nature. Concerning gamete/ embryo donation both non-religious and religious participants expressed a variety of outlooks including objection, agreement, and ambiguity. But the majority of religious participants including Protestants and Sunni Muslims presented their opposition. Their interpretations of these procedures were expressed as "going to the extremes", "a treat for next generation", fulfilling a "huge moral dilemma", "something horrible" and "producing an illegitimated child". In contrast, the majority of Shiite Muslims, who were religiously allowed to use donor procedures, and a few number of Protestant, Catholic and Orthodox participants, despite not being religiously allowed, were keen to carry out gamete/ embryo donation. Non-religious participants mainly asserted that they would choose it as the last resort in circumstances where there is no other choice to conceive. They believed that it would be the single way for experiencing motherhood. Regarding surrogacy, the majority of religious and non-religious infertile women advocated it, although a few Protestants and Sunni Muslims were disagree or uncertain to acknowledge surrogacy.
Conclusion: The findings revealed diverse views of religious and non-religious infertile women pertaining to ART, which indicates that people do not always practice based on religious authorizations, instead they have personal justifications probably affected by social and cultural constructions, which guide them how to deal with their illness. They may or may not obey religious perspectives in the situation where they have a dramatic feeling of desperation. We argue that health professionals' awareness of these various perspectives helps them to encourage patients to initiate discussion on their religious and spiritual concerns regarding ART.

Keywords: Infertility, Religion, Spirituality, ART, Feminist Grounded Theory

P-29: Progestrone for Prevention of Preterm Birth and Improvement Pregnancy Outcomes Among Primiparea of Advanced Maternal Age

Moghtadaei P[1*], Sardari F[1], Latifi M[2]

1. IUMS, Ob & Gyn Department, Tehran, Iran
2. IUMS, Pediateric Department, Tehran, Iran
Email: drmoghtadei@gmail.com

Objective: In many articles, 17aipha-hydroxy progestrone caproate(17p) have been shown to reduced recurrent preterm labor and also in women with short cervix or twin pregnancy.This sudy were undertaken to evaluate wether wuold reduce the preterm birth in advanced maternal age.
Materials and Methods: A randomized ,double-blind , placebo controlled trial in primiparous aged 35 years or more was performed.Patiens were assigned to weekly intramuscular injections of 250 mg of 17P or matching placebo, starting at 16-20 weeks of gestation and ending at 34 weeks.The primary and secondery outcomes were assessed.
Results: Two-hundreds and sixty women were assigned to treatment.Delivery before 37 weeks occured in 27.7% of pregnancies in17P group and 36.5% of patients in placebo group, RR 0.6 (95% CI), and also delivery before 35 weeks was 10.6% vs. 20.7%, RR 0.67 (95% CI) and delivery before 32 weeks was 6.4% vs. 12.2%, RR 0.58 (95% CI). Secondery outcome in infant and other pregnancy outcomes like hypertention, diabetes, IUGR in 17P group were lessthan placebo group.Side effect of injection site in both groups occuring 61% and 58.5% of subjects, respectively (p=0.633), but was mild and limited to injection sites.
Conclusion: Treatment with 17 alpha-hydroxy progestrone caproate can reduce the rate of preterm birth in primiparous advanced aged women.

Keywords: Preterm Birth, Advanced Age, 17Alpha-Hydroxy Progesrtone Caproate

P-30: Estimate of Breast Cancer Risk in Early Stages

Sadeghi M

Isfahan, Iran
Email: m.sadeghi@biol.ui.ac.ir

Objective: Breast cancer is the most malignancy among women and so leading case of female death in middle age. Knowledge of the genes include in this disease and their disorders is so important for treat management. The matrix metalloproteinases (MMPs) have been shown to play important roles in some cancers progression and metastasis. The aim of this study was investigation the role of a single nucleotide polymorphism in the promoter of a major member of this family (collagenases IV) and its effect in breast cancer metastasis.
Materils and Methods: In this study 190 subjects including two groups of controls and patients were detected by PCR-RFLP assay and gel electrophoresis for statistical analysis we used chi square test and SPSS software.
Results: The results showed a positive association between the existence of T allele and breast cancer risk (p=0.0004).
Conclusion: This association can arise from the Over-expression of this gene in the alleles consist of thymine nucleotide and so increase the susceptibility of cells cancer by digestion of growth factors inhibitors and increase separation of the growth factors by this enzyme.

Keywords: Basement Membrane Contacts, Breast Cancer, Matrix Metalloproteinase-9

P-31: A Survey of the Positive Hbsag Frquency Pregnant Women Referring to Health Centres of Ahwaz City

Saadati N[1*], Mohammad Jafari R[2]

1. Community Medicine Department, School of Medicine, Ahwaz Jondi-Shapour University of Medical Sciences, Ahwaz, Iran
2. Obstetrics and Gynecology Department, Ahwaz Jondi-Shapour university of Medical Sciences, Emam khomaini Hospital, Ahwaz, Iran
Email: saadatynasrin@yahoo.com

The Viral infection of Hepatitis B is accounted as an important world wide problem, the connection of which with the gynecology due to the high risk of Vertical transmission from mother to fetus. Chronic infection leading to the potential preventives B Vaccine and immunoglobuline application. The most common reason of pregnancy jaundice is viral hepatitis. The researches showed that probability of preterm labor increased following hepatitis B in third trimester. 90% of infants that contaminated at prenatal period, 50% of children and 1-10% of adults produced chronic Carrier State. This study was carried out to estimate such a frequency of HbsAg was considered by simple sampling method on 120 pregnant women referring to health centers of Ahwaz City from March up December 1998.. The data was collected through completing the questionnaire, inter view as a supplementary tool accompanied with laboratory test results. The research showed that 2 out of 120 specimens were positive HbsAg with no evidence of HBEAg.
Viral hepatitis is the fifth one reason of premature death in world. Our results and other studies in regard to the high Prevalence of chronic Hepatitis B carriers, with attention to transmission from mother to fetus, supports a generalized screening of all pregnant women in concern of HbsAg.

Keywords: HBSAg (Hepatitis B Antigen), HBEAg, Frequency, Pregnant Women, Ahwaz

P-32: Clinical Outcome with Half-Dose Depot Triptorelin is the Same as Reduced-Dose Aaily Buserelin in a Long Protocol of controlled Ovarian Stimulation for ICSI/Embryo Transfer: a Randomized Double-Blind Clincal Trial (NCT00461916)

Soltani F

Tehran UMS, Obstetrics and Gynecology Department, Tehran, Iran

Emal: medlars@gmail.com

Objective: Traditional doses of depot GnRH agonist may be excessive for ovarian stimulation. We compared half-dose depot triptorelin (Group I) with reduced-dose daily buserelin (Group II) in a long protocol ICSI/embryo transfer through a double-blind randomized clinical trial.
Materials and Methods: Controlled ovarian stimulation (COS) was started by a pretreatment with oral contraceptives for 21 days. Then, 182 patients were randomized into two groups of 91. Group I received 1.87 mg triptorelin depot i.m. followed by daily s.c. injections of saline. Group II (reduced-dose protocol) received a bolus injection of i.m. saline followed by daily s.c. injections of 0.5 mg buserelin, which was then reduced to 0.25 mg at the start of human menopausal gonadotrophin stimulation. When transvaginal ultrasound showed at least two follicles of 16–20 mm diameter, HCG was given and ICSI was performed 40–42 h later.
Results: No significant differences were seen in the mean (SD) number of follicles at HCG administration, as our primary outcome [10.3 (4.4) in Group I versus 11.1 (4.2) in Group II, p=0.180, mean difference = 0.86, 95% confidence interval 0.39–2.11]. The other early results of COS, clinical and ongoing pregnancy rates, or early pregnancy loss were also not significantly different between the groups. Group I endured longer stimulation period [11.2 (1.8) days versus 10.6 (1.9), p=0.030].
Conclusion: Clinical outcomes were not significantly different between Group I and Group II.

Keywords: Depot Triptorelin, Daily Buserelin, Controlled Ovarian Stimulation, Long Protocol, ICSI

Genetics

P-33: The Coralation Between Sperm and Leukocyte DNA Integrity by Comet Assay

Babazadeh Z[3*], Nasr Esfahani MH[1,2], Razavi S[3], Sabahi A[3]

1. Embryology Department, Cell Sciences Research Center, Royan Institute, ACECR, Tehran, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran.
3. Anatomy Departement, Isfahan Medical University, Isfahan, Iran
E-mail: mh_nasr@med.mui.ac.ir

Objective: Both endogenous and exogenous factors have been proposed to explain the presence of DNA fragmentation in human spermatozoa. Also exogenous factors can effect leukocyte DNA integrity. The aim of this study was to evaluate whether there is a relation between sperm DNA damage with leukocyte DNA integrity.
Materials and Methods: DNA damage in sperm of unselected group of 62 men undergoing IVF and ICSI treatment was measured by comet assay and SCD and analysis were performed by visual and computer scoring. DNA integrity of leukocyte was only assessed by comet assay
Results and Conclusion: No correlation was observed between the DNA integrity of sperm with that of leukocyte

P-34: GABAA Receptor Subunits in Rat Sperm

Esmaeili A.

Biology Department, Faculty of Science, The University of Isfahan, Isfahan, Iran
Email: abesmaeili@yahoo.com

γ-Aminobutyric acid (GABA) is considered as the predominant inhibitory neurotransmitter in mammalian central nervous systems (CNS). GABAARs are constructed from a family of around 21 different subunits including six alpha (α1-6), four beta (β1-4), four gamma (γ1-4), one delta (δ), one epsilon (ε), one pi (π), one theta (θ), and three rho (ρ1-3) subunits all of which are products of separate genes. The presence of some GABA receptors in sperm prompted us to explore the existence of GABAA receptors in testis and sperm. All experiments were performed on adult Wistar rats. Total cellular RNA was extracted and was reverse transcribed. PCR reactions were performed specific $GABA_AR$ subunits primers. Reactions were carried out as follows: an initial 95 0C denaturation step for 30 second, annealing at 55 ^{0}C for 30 second and extension at 72 ^{0}C for 30 second repeated 40 times. The amplification products were analysed on 2% agarose gels stained with ethidium bromide. Our present results showed that GABAARs composed of α5, β1, β3 and γ1 subunits were expressed in both testis and sperm. These results indicate that, in sperm, GABAA receptors might have functions.

Keywords: GABAAR, RT-PCR, Rat Testis, Sperm

P-35: Down-Regulation MT1 Gene in Testis Tissue After Exposure to Electromagnetic Field

Habibi Roudkenar M[1], Mozafari P[1], Halabian R[1], Mohammadipour M[1], Mohammadi Roushandeh A[2*], Soleimani Rad J[2]

1. IBTO, Research Center, Tehran, Iran
2. Tabriz Medical University, Anatomy Department, Tabriz, Iran
Email: dinachal@yahoo.com

Objective: Because of conditions provided by the modern life, the human being is exposed to EMF. Appliance such as television, computer, mobile set, and power producting systems have important role in human life are the source of EMF. Epidemiological and

experimental studies have shown the adverse effect of EMF on biological systems. It is proved that after EMF exposure reactive oxygen species are produced in different tissues. In other hand it is clear that metalothionine1 and 2(MT1 and MT2) act as antioxidants. The aim of this study is to investigate expression of MT1 and MT2 in testis tissue.
Materials and Methods: In this study, 10 bulb/c mice were exposed to 3MT EMF for 2 months, 4 hours/day. After 2 months, these mice were sacrificed by cervical dislocation and testis removed. This tissue was fixed by formalin 10% and tissue passage processing is performed. Slides were stained by hematoxiline-Eosin (H&E). Number of leydic and primary spermatocytes cells and diameter of basal lamina were measured by light microscope. For Real-time RT-PCR, Total RNA from testis tissues was extracted by Trizol reagent the quantity and quality of RNA were determined by spectrophotometry and electrophoresis, respectively. Reverse transcription was performed by SuperScript III reverse transcriptase with 1 µg of total RNA followed by DNaseI treatment and heat inactivation. Expression of MT1 and MT2 was determined by semi quantative and real time-PCR
Results: The light microscopic study of seminiferous tubules showed that basal lamina was thickend. Number of Primary spermatocytes was increased compared to control group. Study on interestitial space showed that number of leydic cells increased. Real-Time PCR result showed that MT1was down- regulated in both semi-quantative and real time-PCR but MT2 expression did not changed.
Conclusion: Testis is one of the organs that express metalothionine in high level. It is also one of the sensitive organs in exposure to electromagnetic filed. Taken together, down regulation of MT1 might account for some consequences of electromagnetic exposure including infertility. However, further and complementary studies are required in this regards.

Keywords: MT1, MT2, EMF, Mouse

P-36: Current Situation on Study of Genetic Aspects of Infertility in Azerbaijan

Malikova Ahmadov I

Khazar, Cell biology and Genetic Department, Baku, Azerbaijan
Email: Lmelikova2002@yahoo.com

Objective: Since 1990 have no information about frequency of genetic disorders among infertile couples in Azerbaijan. Last convincing data was reported that, its approximately 25 to30 percent infertile couple among the reproductive family. The rate of genetic disorders in infertility couples was nearly 5-7%. These results were validated using classical karyotypes, which made after mitotic arrest of dividing cells followed by a banding method. This was sufficient for the study of all numerical and most structural constitutive abnormalities. These are not discuses about female and male factors of infertility and others. The expansions of genetic investigation are open new avenues of understanding of various previously unknown conditions and associations of modern genetics. Modern genetic, in general, means modern laboratory, as well as its clinical offshoot medical genetics which have gone through a huge development in past decades.
Materials and Methods: Nowadays the genetic diagnosis in case of infertility became more and more important. With the introduction of IVF (in vitro fertilization) and other forms of assisted reproduction, it has become possible to correct genetic forms of infertility and to diagnose the resulting early embryos. Currently genetic services including laboratories are established in "Cell biology and genetic research center" Khazar University
Results: Last convincing data was reported that, its approximately 25 to30 percent infertile couple among the reproductive family. The rate of genetic disorders in infertility couples was nearly 5-7%. These results were validated using classical karyotypes, which made after mitotic arrest of dividing cells followed by a banding method. This was sufficient for the study of all numerical and most structural constitutive abnormalities. These are not discuses about female and male factors of infertility and others.
Conclusion: Thus, frequency, prevalent type of genetic disorders among the infertile couples, female and male genetic factors in infertility will discussed.

Keywords: Infertility, Reproductive Health, Genetic Disorder

P-37: Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2

Mirsaidi JZ[1]
Tabriz Medical University, Tehran, Iran
Email: jeyranz@gmail.com

Objective: Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2.
Materials and Methods: This is a systematic review article that involved 29 articles about Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2 (from indexes medicos) of 1985 until 2008 and search of many related topics.
Results: A growth factor receptor gene,5,6,7 human epidermal growth factor receptor (HER2), is amplified in 25 to 30 percent of breast cancers and in these cases the encoded protein is present in abnormally high levels in the malignant cells.8,9 Women with breast can-

cers that overexpress HER2 have an aggressive form of the disease with significantly shortened disease-free survival and overall survival.8,9,10,11,12 Laboratory studies indicate that amplification of HER2 has a direct role in the pathogenesis of these cancers, 13, 14, 15, 16, 17 thereby providing investigators with an opportunity to target a therapeutic agent directly against the alteration. Several murine monoclonal antibodies against the extracellular domain of the HER2 protein were found to inhibit the proliferation of human cancer cells that overexpressed HER2, both in vitro and in vivo.18-20 To minimize immunogenicity, the antigen-binding region of one of the more effective antibodies was fused to the framework region of human IgG21 and tested against breast-cancer cells that overexpressed HER2 in vitro and in vivo.21,22 This antibody, called trastuzumab, inhibited tumor growth when used alone4 but had synergistic effects 20, 22, 23, 24 when used in combination with cisplatin and carboplatin, 20, 23 docetaxel, 24 and ionizing radiation25 and additive effects when used with doxorubicin, cyclophosphamide, methotrexate, and paclitaxel 22, 23, 24, 25, 26.

Conclusion: Phase 1 clinical trials showed that the antibody is safe and confined to the tumor (unpublished data). Subsequent phase 2 trials demonstrated that many women with HER2-positive metastatic disease who had relapsed after chemotherapy had a response to trastuzumab27,28; as suggested by the preclinical data, the efficacy of trastuzumab when given with chemotherapy was superior to its effectiveness when used alone.28,29 We report the results of a phase 3 trial in which women with cancers that overexpressed HER2 who had not previously received chemotherapy for metastatic disease were randomly assigned to receive either chemotherapy alone or chemotherapy plus trastuzumab. The primary end points of the study were the time to disease progression and the incidence of adverse effects. Secondary end points were the rates and the duration of responses, the time to treatment failure, and overall survival.

Keywords: Chemotherapy, Monoclonal Antibody, HER2, Metastatic Breast Cancer

P-38: Down Regulation of Lcn2 Gene After EMF Exposure in Mouse Testis Down Regulation of Lcn2 Gene After EMF Exposure in Mouse Testis

Mohammadi Roushandeh A[1*], Habibi Roudkenar M[2], Halabian R[2], Mozafari P[2], Soleimani Rad RJ[3], Sadeghzadeh Oskuei B[4]

1. Tabriz Medical University, Anatomy Department, Tabrioz, Iran
2. IBTO, Research Center, Tehraniran
3. Tabriz Medical University, Anatomy Department, Tabriz, Iran
4. Tabriz Medical University, Anatomy Department, Tabriz, Tehran
Email: dinachal@yahoo.com

Objective: Neutrophil gelatinase-associated lipocalin (NGAL), comprise a group of small extracellular proteins with a common b-sheet-dominated 3-dimensional structure. In the past, the predominant role of lipocalins was assumed to be to act as transport proteins and, for several members, this is likely to be an important function. Recently it is suggested that LCN2 has role in ROS scavangering. It is proved that Electromagnetic field could produce ROS in different tissues. Expression of LCN2 in such condition in testis tissue and its role in reproduction was investigated in this study.

Materials and Methods: In this study, 10 bulb/c mice were exposed to 3MT EMF for 2 months, 4 hours/day. After 2 months, the mice were sacrificed by cervical dislocation and testis removed. This tissue was fixed by formalin 10% and tissue passage processing is performed. Slides were stained by hematoxiline-Eosin (H&E). Number of leydic and primary spermatocytes cells and diameter of basal lamina were measured by light microscope. For Real-time RT-PCR, Total RNA from testis tissues was extracted by Trizol reagent the quantity and quality of RNA were determined by spectrophotometry and electrophoresis, respectively. Reverse transcription was performed by SuperScript III reverse transcriptase with 1 µg of total RNA followed by DNaseI treatment and heat inactivation. Expression of Lcn2 was determined by semi quantative and real time-PCR.

Results: The light microscopic study of seminiferous tubules showed that basal lamina was thickend. Number of Primary spermatocytes was increased compared to control group. Study on interestitial space showed that number of leydic cells increased. Real-Time PCR result showed that Lcn2 was down- regulated in both semi-quantative and real time-PCR.

Conclusion: Testis is one of the organs that express Lcn2 in high level. It is also one of the sensitive organs in exposure to electromagnetic filed. Taken together, down regulation of Lcn2 might account for some consequences of electromagnetic exposure including infertility. However, further and complementary studies are required in this regards.

Keywords: EMF, LCN2, Testis, ROS

Stem Cells

P-39: hLIF and bFGF Effects on Maintenance of Horse Embryonic Stem Cells Pluripotency

Abavisani A[1*], Baghbanzadeh A[1], McKinnon A[2], Tecirlioglu RT[3], Guo J[3], Trounson AO[4]

1. University of Tehran, Physiology Department, Tehran, Iran
2. Goulbourn Equine Hospital, Shepparton, Australia
3. Monash University, MISCL, Clayton, Australia
4. CIRM,California, USA

Email: abavisani@ut.ac.ir

Objective: Our aims were to derivate horse ES cell lines by repeated passage of ICM cells and assess hLIF and bFGF effects on maintenance of their pluripotencies
Materials and Methods: A basic medium was prepared for the culture of ICMs and horse embryonic stem cells using KODMEM supplemented with 10% FBS, 0.1 mM Non-essential Amino Acids, 2 mM L-glutamine, 1% Insulin-Transferrin-Selenium, 100 µg/ml of streptomycin, 100 units/ml of penicillin and 0.1mM 2β-mercaptoethanol on γ-irradiated MEFs. ICM cells were recovered mechanically from day-7 blastocysts and cultured in basic medium on feeder cells. The culture medium was changed every day after first passage and the passage was performed by mechanical dissociation with a needle every 6-8 days until passage 15. The putative horse ESCs were cultured in the basic medium supplemented with human leukemia inhibitory factor (hLIF 40ng/ml) only, basic fibroblastic growth factor (bFGF 4ng/ml) only, or bFGF plus hLIF with MEF feeder cells. Colony morphology was evaluated, continuously. Finally, for determination of cell pluripotency, the hESCs were analyzed for markers of pluripotency. Immunostaning of the putative horse ESCs was done for Oct4, SSEA-1, GCTM-2, TRA1-60 and TRA1-81.
Results: Derived ICMs, from day-7 blastocysts, were cultured with basic medium on MEF feeder cell monolayer. They grew as flat colonies including cells with different morphology (P0). Different morphological cells were transferred for next passages separately (P1). Cells in basic medium only, and basic medium added LIF could keep colony form similar to each other until passage 15. Although the cells in basic medium containing bFGF could produce some colonies in early passages, they could not maintain their morphology in later passages. Those colonies which were plated in basic medium added hLIF and bFGF did not grow after a few passages. After 15 passages, immunohistochemistry of the putative horse ESCs showed that some cells in the colonies were positive for Oct4, SSEA-1, GCTM-2, TRA1-60 and TRA1-81. These results indicated that those cell colonies were not completely purified embryonic stem cells, but some of them were still pluripotent.
Conclusion: Our study show the primary horse ESCs are able to self-renew when they are cultured in the basic medium on γ-irradiated MEFs. These results also indicated that cell colonies were not completely purified embryonic stem cells, but some of them were still pluripotent. ESC-like cell morphology of horse putative ESCs were well maintained in the basic medium supplemented with or without hLIF. This result suggests that hLIF is neither prerequisite nor negative for maintenance of horse ESCs; bFGF seem to be negative for maintenance of horse ECSs. The combination of LIF and bFGF is unable to improve the culture condition. Thus, the new factors need to be investigated further to maintain horse ESCs in purified population

Keywords: Embryonic Stem Cells, Horse, hLIF, bFGF, MEF

P-40: Isolation, Culture and Characterization of Human Synovium Derived Mesenchymal Stem Cells

Darvishi M[1, 2]

1. Pasture Institute, Tehran, Iran
2. Payame Noor University, Tehran, Iran
Email: darya9359@yahoo.com

Objective: Recently, it has been demonstrated that Mesenchymal stem cells (MSCs) which are isolated from various tissues have different potentials in differentiation and proliferation and for this reason it is necessary to isolate these cells from various kinds of origins in order to using in clinical demands. The present study has been done to investigate the possibility of isolation, culture and characterization of human synovium –derived mesenchymal stem cells.
Materials and Methods: Samples (200-300 mg) were provided from synovium tissue and subsynovial inner layer of medial joint capsule of patients who had knee surgical operations. Obtained samples were homogenized, enzymatically minced with collagenase D and passed through 70 µm nylon filters and then nucleated cells were isolated and cultured. Isolated cells were identified with morphological, immunocytochemistery and differentiation tests
Results: The isolated cells in this study showed fibroblast-like morphology and like the mesenchymal stem cells have high proliferation capacity. In immunocytochemical studies they were positive for CD73, CD105 and STRO-1 antigens. After induction toward osteogenic and adipogenic lineages and specific staining for each lineage it was revealed that isolated cells were potent in differentiation into mentioned lineages.
Conclusion: These results suggest that synovium tissue which is discarded in most knee operations can be used for cell therapy and tissue engineering protocols as an enrichment source of potent mesenchymal stem cells.

Keywords: Mesenchymal Stem Cells, Synovium Tissue

P-41: Assessment of Cryopreserved Cord blood Units in Private Royan Cord Blood Bank

Ebrahimi M[1, 2*], Zarrabi M[2], Samimi S[2], Mohammad M[2], Etedali Kh[2], Jan Zamin E[2], Ahmadi MH[2], Khosh Akhlagh A[2], Shayan N[2], Zandieh T[2], Abron S[2,3], Aghdami N[1], Kasaeian M[2]

1. Stem Cells Department, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran
2. Royan Cord Blood Bank, Royan Stem Cell Technology Co, Tehran, Iran

3. Heamtology Department, Faculty of Medicine, Tarbiatmodares University, Tehran, Iran
Email: Ebrahimi@royaninstitute.org

Objective: Cord blood (CB) is a unique product, rich in haemopoietic stem cells (HSC), that is currently used in the transplantation setting to restore haemopoiesis in patients suffering from malignancies, bone marrow (BM) failure disorders and inherited metabolic and immunological disorders. Royan Cord Blood Bank (RCBB) was established in 2003 and it has cryopreserved about 4000 units according to parents submission.
Materials and Methods: During delivery, cord blood was collected in cord blood collection bag with anticoagulant solution, and was shipped to RCBB in transporter flask. Cord Blood units were processed and cryopreserved in presence of DMSO:Dexteran (10%: 1%) solution in controlled-rate freezer and were storage in liquid nitrogen.The number and viability of WBC, BFU-E, CFU-GM, CFU-MIX, and the absolute CD34+ cell count were assessed pre and post cryopreservation.
Results: Based on stringent mother selection criteria, 2227 UCB units were collected from July 2005 to March 2006. All data from donors save in electronic databank and worksheet. Median values for specific Parameters pre cryopreservation were as follows: Median of volume : 90.31±29.22 ml (5% of units were processed <50 ml), TNC Count per unit: 346.9±193.59×106 (16.56%≥500×106), Viability: 98.3±1.17% and % CD34+ Cells was %0.46±0.20.
A total 50 units were thawed after 6-12 months. Evaluation of viability for fresh were 97.72± 6.17 and freezed 84.28±6.04. colony forming assay for fresh and freezed cells were 34.3±18.3 and 34.811.71±, respectively and %CD34 cells were %0.97±0.34 versus %0.76±0.37 pre and post cryopreservation , respectively.
Conclusion: These data show that do not significantly affect the clonogenic potential and CD34+ Cells during the long term storage in liquid nitrogen. The results obtained during this initial period are encouraging and indicate that the UCB banking at RCBB will help to improve already existing hematopoietic cell transplant programs in Iran. The experience generated at RCBB may be helpful to other institutions, particularly those in developing countries.

P-42: Systemic Transplantation of Mesenchymal Stem Cells Can Reduce Cognitive and Motor Deficits in Rats with Unilateral Lesions of the Neostriatum

Edalatmanesh MA, Matin MM, Neshati Z*, Bahrami AR

Biotechnology Research Center, Biology Department, Mashhad University, Mashhad, Iran
Email: neshatizeinab@gmail.com

Objectives: Huntington's disease is an inherited neurodegenerative disorder, which usually happens in third or fourth decades of life. Stem cell therapy is one of the approaches for HD treatment.
Materials and Methods: Since mesenchymal stem cells (MSCs) have the ability to migrate into the lesion site, we transplanted rat bone marrow derived- MSCs intravenously, following unilateral intrastriatal lesion made by quinolinic acid (QA) in Wistar rats. QA administration caused widespread neuropathological deficits similar to those found in Huntington>s disease, including impairment in motor and cognitive function. Animals receiving MSCs, exhibited significant improvement in motor and cognitive performance compared to sham group as judged by apomorph-ineinduced rotations, beam walk, cylinder and hang wire tests.
Results: These results indicate that systemic transplantation of MSCs can significantly reduce the behavioral abnormalities of these animals.
Conclusion: This method of systemic injection has a great advantage over invasive surgical techniques for transplanting the cells at the lesioned site.

Keywords: Huntington's Disease, Mesenchymal Stem Cells

P-43: Differential Expression of Glutathione S-TransFerases P1-1 and A1-1 at Protein and mRNA Levels in Hepatocytes Derived from Human Bone Marrow Mesenchymal Cells

Esmaeli Sh[1*], Allameh A[1*], Kazemnejad S[1], Soleimani M[2]

1. Clinical Biochemistery Department, Tarbiat Modares University, Tehran, Iran
2. Hematology Department, Tarbiat Modares University, Tehran, Iran
E-mail: allameha@modares.ac.ir

Differentiation of bone marrow mesenchymal stem cells (hBMSCs) into hepatocytes in vivo or in vitro is well established. However, so far few studies have been carried out on the detoxification potential of these cells. Glutathione S-transferases (GSTs;EC 2.5.1.18) are a family of isoenzymes which are widely distributed in animal tissues, their physiological function is detoxification of xenobiotics and drugs. In this study, the expression of two major isosyms of glutathione S-transferases namely GST A1-1 and GST P1-1 was evaluated in hepatocyte-like cells derived from hBMSCs at mRNA and protein levels. Also level of GSH evaluated in mesenchymal stem cells before and after differentiation.
Human bone marrow were isolated and cultured in vitro. Hepatic differentiation was performed using 2-step protocol employing the hepatocyte growth factors, dexamethasone and oncostatin M. Differentiated cells were examined for their ability to express liver specific markers (albumin and α- fetoprotein) using reverse transcription polymerase chain reaction (RT-PCR) and immunocytochemistry. Then expression of glutathione

S-transferases (GSTs) clases A1-1 and P1-1 were estimated in hepatocyte-like cells derived from MSCs using western blotting and RT-PCR. Moreover GSH level was compared in hepatocyte-like cells and their progenitpr stem cells.
Preliminary results revealed that GST specific activity using 1-chloro-2, 4-dinitrobenzene as the substrate, was markely increased (~3 folds) in differentiated cells when compared with MSCs. Whereas GSH level in these cells is decreased during differentiation of hepatocyte-like cells. GST P1-1 was expressed at protein level in both MSCs and hepatocyte-like cells as judged by western blotting technique, whereas expression of GST A1-1 protein was limited to differentiated cells. These findings were further confirmed by measuring GST A1-1 and GST P1-1 specific mRNA level in MSCs before and after differentiation. This data show unlike GST P1-1, the expression of GST A1-1 commence to development of hBMSCs to hepatocyte-like cells.
Cumulative results indicated that hepatocyte-like cells derived from hBMSCs acquired potential of resistance to toxic agents with expression of sufficient levels of GSTs enzymes and GSH.

P-44: Expression of Glutathione S-Transferses A1-1 and P1-1 in Human Bone Marrow Mesenchymal Stem Cells

Esmaeli Sh[1*], Allameh A[1], Gaderi M[1], Kazemnejad S[2], Soleimani M[3]

1. Tarbiat Modares, Clinical Biochemistery, Tehran Iran
2. Tarbiat Modares, Clinical Biochemistry, Tehran Iran
3. Tarbiat Modares, Clinical Hematology, Tehran, Iran
Email: allameha@modares.ac.ir

Objective: Human bone marrow mesenchymal stem cells (hBMSCs) are multipotent adherent cells able to differentiate to different cell lineages. MSCs are exposed to various drugs and xenobiotic compounds. However very little information is available on their ability to detoxify foreign compounds. The aim of this study was to find out the activity of two major GST classes involved in detoxification mechanism
Materials and Methods: Bone marrow aspirates (10 ml) were obtained from iliac crests of human donors (n=10) ranging in age from 19 to 32 years. The aspirates were diluted with phosphate buffer saline (PBS). Cell solution was gently overlaid on the Ficoll-Hypaque (D=1.077g/ml) to eliminate unwanted cell types that were present in the marrow aspirate. Mononuclear cells were recovered from the gradient interface and washed with PBS after centrifugation at 400g for 30 min at room temperature (RT). The isolated mononuclear cell layer were then washed in PBS, resuspended in growth medium containing DMEM-low glucose supplemented with 15% FBS, 2 mM glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin and plated in polystyrene plastic 75-cm2 tissue-culture flasks. The cell cultures were maintained at 37°C in a humidified 5% CO2 incubator. Following 3 or 4 days of incubation, the non-adherent cells were washed away leaving behind the adherent cell population that was growing as fibroblastic cells in clusters. When cells reached 70–90% confluence, cultures were harvested with 0.25% trypsin-EDTA solution. Then the cells were processed for measurement of GST specific activity using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Moreover the cells were processed for detection of two major GST classes using western blotting and. reverse transcription polymerase chain reaction (RT-PCR) techniques.
Results: The results showed that cellular GST specific activity was found to be 78 2/8 nmol/min/mg protein in MSCs. Based on western blotting technique using polyclonal antibodies, we showed that MSCs could express GSTP1-1 at protein levels. In contrast, GSTA1-1 was not detected in these cells. Also expression of GSTA1-1 and GSTP1-1 at the mRNA level showed that MSCs are capable of expression GSTP1-1, but failed to express GSTA1-1.
Conclusion: In conclusion, these results showed that GSTs are sufficiently expressed in human MSCs to cope with xenobiotic compounds. However probably different classes of enzymes undergo developmental changes during growth and differentiate to different cell lineages

Keywords: Mesenchymal Stem Cells, GSTs

P-45: Characterizing Endothelial Cells Derived From the Murine Embryonic Stem Cell Line CCE

Fathi F

Kurdistan University, Anatomy Department, Sanandaj, Iran
Email: alasvand50@yahoo.com

Objective: Embryonic stem cells (ESC) are defined by two main properties of self-renewal and their multipotency to differentiate into virtually all cell types of the body, including endothelial cells. ESCs have been widely regarded as an unlimited source of cells in regeneration medicine and also an ideal in vitro model to investigate complex developmental processes. Here, we report a simple and efficient in vitro model to derive a nearly pure population of endothelial cells from a murine ESC line.
Material and Methods: CCE ES cells are exposed to alpha-MEM medium containing 10% FBS for 4 days and then cultured in endothelial basal-2 medium containing vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and 2% FBS for 42 days.
Results: The cells acquired a relatively uniform endothelial cell morphology and were able to propagate

and expand in culture. When murine ES cell–derived endothelial cells (MESDECs) were cultured on Matrigel and incubated for 48 h, vessel-like tube structures consisting of CD31 (PECAM-1) or BS-1 immunoreactive cells were developed. Immunocytochemistry and RT-PCR analyses revealed that MESDECs express endothelial cell-specific marker proteins such as Flk-1, PECAM-1, Tie- 1, and Tie-2, in which the expressions persist for long periods of time after differentiation. The cells were also capable of taking up acetylated low-density lipoprotein (LDL) in culture. .
Conclusion: Our data suggest that MESDECs could provide a suitable in vitro model to study molecular events involved in vascular development and open up a new therapeutic strategy in regeneration medicine of cardiovascular disorders

Keywords: Embryonic Stem Cells, Endothelial Cells, Tube Formation, Differentiation

P-46: Transfection of Mouse Neural Stem Cells by Electroporation Method

Fathi F

Kurdistan University, Anatomy Department, Sanandaj, Iran
Email: alasvand50@yahoo.com

Objective: Over the past two decades, the ability to transfer genes into stem cells has raised hopes towards the feasibility of using cell-based gene therapy approaches to provide long-term therapeutic impacts. Different viral and non-viral strategies have been proposed to transfer the exogenous genes into stem cells. Non-viral strategies have several advantages over viral ones. Electroporation is one of the most promising non-viral strategies which have been used to introduce exogenous genes into a variety of stem cells. Neural stem cells (NSCs) are among adult stem cells which hold great promise for treatment of neurodegenerative diseases. In this project, we evaluated the efficiency of electroporation to transfer exogenous genes (GFP & BDNF) into NSCs isolated from SVZ of mouse brain
Materials and Methods: Extraction of the amplified pEGFP-c1 and pCDNA3-hBDNF plasmids was done by endotoxin-free maxiprep kit (Qiagene, USA). Electroporation was done using muliporator device (Eppendorf, Germany). Then, different electroporation parameters, including cell number, plasmids concentration, required voltage and voltage time were optimized for transfer of these two genes into the mouse neural stem cells. G418 antibiotic was used for stabilization of transgenes expression. Inverted Fluorescent microscope was used for detection of GFP expression and RT-PCR was used for detection of BDNF over expression.
Results: we noticed that using of hypo-osmolar buffer for 15 minutes, administration of 600 V for 100 µs and 30µg Plasmid with 800000 cells would yield to the best result, in which, surprisingly, more than 98% of the obtained cells could express GFP, 24 hours after electroporation. Expression of GFP and over-expression of BDNF maintained up to two months, using 300 µg/ml G418 antibiotic. Finally, we obtained a cell line of NSCs which could constantly express GFP and BDNF genes. The cell line is potentially an attractive source of donor cells in treatment of neurodegenerative diseases in animal models
Conclusion: Our results demonstrate that Mouse NSCs could be transfected with exogenous genes, easily and efficiently by means of electroporation method, thus provide a promising source for development of stem cell-based gene therapy strategies.

Keywords: Neural Stem Cells, Electroporation, GFP, BDNF

P-47: Quantification of Morphological Changes of Mesenchymal Stem Cells by Cyclic Stretch

Ghazanfari S[1*], Tafazzoli-Shadpour M[1], Shokrgozar MA[2]

1. Amirkabir University, Biomedical Engineering, Tehran, Iran
2. Pasteur Institute, National Cell Bank, Tehran, Iran

Objective: Mesenchymal stem cells (MSCs) can be expanded and differentiated to several lineages of mesenchymal tissues such as adipocytes, osteocytes, cardiomyocytes, fibroblasts, chondrocytes, neurons, tenocytes and bone-marrow stromal cells. In addition to growth factor, mechanical stimuli such as cyclic stretch contribute to differentiation of stem cells. This study was performed to study effects of cyclic stretch on cultured MSCs. Mechanical stimuli are critical to morphological changes, development, regeneration, differentiation and pathology of mesenchymal tissues. Tissues and cells are constantly exposed to a complex physico-mechanical environment in vivo. To obtain successful engineered tissues in vitro, simulation of environmental conditions is required
Materials and Methods: To study effects of mechanical load on morphology of stem cells, cyclic strain is applied on cultured stem cells by use of a designed stretch device capable of operation inside an incubator with different load amplitude, frequency and number of cycles. MSCs cultured on collagen coated silicon membrane are subjected to cyclic loading. The tests are performed for 5% and 15% load amplitudes with 1Hz frequency and 1-4 hour durations. Topological parameters of cellular images before and after tests are calculated by development of an image processing code.
Results: Cells show change in cell orientation from randomly orientated before test to an align network after loading. The orientation angle tends to be perpendicular to the strain axis to minimize the amount of stress applied on cell bodies. Comparison of MSCs

images before and after test indicates alignment, rearrangement and elongation of cells after cyclic stretch loading. Results demonstrate statistically significant changes in cell topology due to mechanical stretch. Elevation of number of cycles leads to further alignment and elongation.
Conclusion: It is concluded that tensile loading influences cell morphology and alignment, a mechanism for functional adaptation, structural regulation and differentiation. Such loading affects functionality of stem cells, and hence, can be used for achievement of differentiated target cells with different functionalities in tissue engineering.

Keywords: Mesenchymal Stem Cells, Cyclic Strain, Cell Morphology

P-48: Stable Expression of Green Fluorescent Protein in Mesenchymal Stem Cell

Halabian R[1*], Habibi Roudkenar M[1], Amirizadeh N[1], Mozafari P[1], Mohammadi Roushandeh A[2]

1. IBTO, Research Center, Tehran, Iran
2. IBTO, Research Center, Tabriz, Iran
Email: mhr376@yahoo.com

Objective: Green fluorescent protein (GFP) from the jellyfish Aequorea victoria has vaulted from obscurity to become one of the most widely studied and exploited proteins in biochemistry and cell biology. In this study GFP Transfected to Mesanchymal stem cell and a cell line expressing GFP was established.
Materials and Methods: Mesanchymal stem cell was isolated from bone marrow and transfected with pEGFP-N1 vector using fugene HD. Mesanchymal stem cell expressing the pEN1-GFP construct were selected in a medium containing Geneticin for at least 14 days. Several stable clones were generated by dilution of the cells and culture in 96 well culture plates. The expression of GFP was determined by RT-PCR and florescent microscope.
Results: Stable clones expressed GFP mRNA as determined by RT-PCR . GFP was also observed under florescent microscope indicating expression of GFP protein.
Conclusion: Here for first time we stably expressed GFP in mesenchymal stem cell . Expression of GFP would be useful as a marker for gene expression and protein targeting in mesenchymal stem cell.

Keywords: Green Fluorescent Protein (GFP), Mesan-Chymal Stem Cell, Stable Transfection

P-49: Neurogenic and Mitotic Effects of Dehydroepiandrosterone on Neural-Competent Marrow Mesenchymal Stem Cells

Hashemi E[1*], Zare N[2], Kazemi S[2], Baharvand H[2], Tavallaei M[3]
1. Jahad Daneshgai Shiraz, Genetic Department, Tehran, Iran
2. Cell Sciences Research Center, Royan Institite, ACECR, Tehran, Iran
3. Baqiatallah Medical University, Biotechnology, Tehran, Iran
Email: e56hashemi@gmail.com

Objective: In this study for first time established that DHEA could enhance the neural differentiation rates of neural-competent bone marrow mesenchymal stem cells (MSCs).
Materials and Methods: First the MSCs differentiated into neural-like stem cells by bFGF,EGF (sigma) with or no DHEA (fluka) and only DHEA. One day next neurospheres complete ,after 7 days the cells plated and differentiated into neural cells by RA(sigma), DHEA, RA+DHEA and no treatment. After 12 days the cells prepared for molecular tests.
Results: Flow cytometry analysis of tubulin-III and Tau positive cells revealed that the percentages of these cells were increased significantly for two markers following presence of DHEA treatment in both stages. Moreover, Westren blott analysis revealed that tubulin-III protein was strongly induced by DHEA. The expression of neuronal specific genes such as Isl-1, Tubulin III, Pax6, and Nestin were detected by RT-PCR analysis. Moreover, BrdU incorporation was increased significantly after DHEA induction
Conclusion: These results have presented evidence that DHEA exert on neural-competent MSCs to induce expression of a comprehensive set of genes and proteins that define neural cells. Moreover, DHEA induced the division of neural-competent MSCs and by this way increased the number of the cells with major characteristics of neuronal cells. To our knowledge, this is the first report that DHEA can induce division and differentiation of MSCs into neurons in vitro and provides a better insight into the treatment of a wide variety of neurologic diseases by MSCs.

Keywords: Dehydroepiandrosterone, Mesenchymal Stem Cells, Neural Differentiation

P-50: Effects of Vitreous Humour on Growth and Differentiation of the Rat Mesenchymal Stem Cells (rMSCs) and Human NTERA2 Cells

Jian Bagherpoor A*, Bahrami AR, Matin MM, Edalatmanesh MA

Biotechnology Research Center, Biology Department, Mashhad, Iran
Email: ajbagherpoor@yahoo.com

Objectives: Two main characteristics of all types of stem cells are their potency for differentiation and self renewal capacity. There are significant attentions to find the conditions and agents which govern these behaviours of the stem cells. It is very well documented that retinoic acid (RA) reduces cell growth rate by in-

duction of cell differentiation in certain conditions and cell lines. On the other hand hyaluronic acid (HA) is known for its growth induction on cultured cells. A natural source of HA, rabbit vitreous humour (VH), was previously shown to promote wound repair in model animals.
Materials and Methods: In search for its possible mechanisms, VH extract was tested on the cultured stem cells.
Results: The cellular and molecular markers (A2B5, Oct4, Sox2) changes showed that VH and possibly HA interferes with differentiating effect of RA.
Conclusion: This reagent may affect cell proliferation and tissue regeneration by inhibition of cell differentiation.

Keywords: Differentiation, Proliferation, Stem cells, Vitreous humour

P-51: Evaluation of Mesenchymal Stem Cells Homing in Bone Marrow After Transplantation in Healthy and Irradiated Rats by PCR Rechnique.

Kargar S[1, 2]

1. Pasture Institute, Tehran, Iran
2. Payame Noor University, Thran, Iran
Email: kargar_85@yahoo.com

Objective: According to the hypothesis which expresses a supporting role of bone marrow mesenchymal stem cells (MSCs) for hematopoietic stem cells (HSCs), many studies have been focused on therapeutic effects of mesenchymal stem cells. This study has been done in order to investigate the possibility of homing and tracing of male rat bone marrow derived-MSCs after allograft transplantation in irradiated and healthy female bone marrow rats.
Materials and Methods: MSCs were isolated and cultured from femoral bone marrow of male rats and identified with morphological observations, differentiation tests and immunocytochemistry stainings. These obtained cells were injected to tail veins of the healthy and irradiated (7Gy) female rats, according to two timetables and two doses. Then in defined time gaps after injection, transplanted rats were killed and their bone-marrow MSCs were isolated and cultured. The DNA of cultured cells were extracted and was amplified by PCR with specific primers for chromosome Y (SRY), and finally PCR products were analyzed on gel electrophoresis.
Results: Phenotypic analysis of MSCs were characterised by immunocitochemistry and differentiated tests. PCR results for the healthy and irradiated rats in one used dose and in all time gaps after transplantation were negative .
Conclusion: These results suggest that after allograft transplantation of MSCs in healthy and irradiated rats, they are unable to detectable homing in bone marrow. This might be the result of trapping of MSCs in other organs or low number of injected MSCs. These results suggest that the better understanding of MSCs behavior in vitro and in vivo is needed to develop strategies for therapeutic applications by these cells.

Keywords: Mesenchymal Stem Cells, Homing, Cell Therapy, PCR

P-52: Profile Analysis of Peroxisomal Marker Genes Expression in Comparision with OCT4 and Nanog During Neural Differentiation of P19 Cells

Mojbafan M[1*], Ghaedi K[1], Razavi S[2], Tanhaie S[3], Karamali F[3], Rabiee F[3]

1. Cell Sciences Research Center, Biology Department, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
2. Medical University of Isfahan, Anatomy Department, Isfahan, Iran
3. Royan Institute, Isfahan Campus, Stem cell Department, Isfahan, Iran

Objective: Peroxisomes are ubiquitous organelles in eukaryotes that participate in the metabolism of fatty acids and other metabolites; they also play important roles in neurogenesis and biogenesis. In order to investigate peroxisome biogenesis profile during neurogenesis, We have performed to set a semi quantitative analysis for two peroxisomal genes expression (Catalase and PEX3) comparing with stem cell marker genes (Oct4 and Nanog) and a house keeping gene (β-actin) in P19 cells. In this project we have used P19 cells which are suitable progenitor cells for the study of neurogenesis. Moreover two different peroxisomal genes were selected to chase both kinetics of peroxisomal matrix protein synthesis (Catalase) and peroxisomal membrane protein biogenesis (Pex3p). Total RNA extracted from P19 cells and were used for cDNA synthesis at the next step.
Materials and Methods: Using specific primers a part of Oct4, Nanog, β-actin, PEX3 and Catalase cDNAs were amplified by RT-PCR for quantitative analysis.
Results: Various parameters were changed to optimize the PCR profiles for each gene for further assessments. Here we describe the conditions which we used in detail.
Conclusion: PEX3 band was observed sharply and amplified at PCR conditions of annealing temperature of 55.1°c, we got β-actin band at 51°c to 55°c degree for annealing. For PCR of Catalase annealing temperature of 52.7°c was used, Nanog band was at 66°c and Oct4 band was observed at 51°c to 57°c.

Keywords: Stem Cell, Naong, Oct4, PEX3, Peroxisome

P-53: Intracellular Sorting Analysis of murine PeP in P19 Cells

Nasr Esfahani MH[1], Tanhaiee S[1], Karbalaiee K[1], Ostadsharif M[1], Ghaedi K[2], Parivar K[3*], Ostadsharif M[3]

1. Cell Sciences Research Center, Biology Department, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
2. University of Isfahan, Biology, Isfahan, Iran
3. Sciences and Researches Campus, Biology, Tehran, Iran
Email: maryam.ostadsharif@gmail.com

Objective: Peroxisomes are near-ubiquitous organelles of eukaryotic cells which perform a range of functions. The common metabolic activities of peroxisomes are the oxidation of fatty acids and generation and removal of hydrogen peroxide, but peroxisomes have also been implicated in β-oxidation of aromatic and cyclic compounds, synthesis of plasmalogens, metabolism of purines and pyrimidines, and catabolism of polyamines, D-amino acids and methanol. One of the peroxisomal matrix proteins which have already been cloned termed peroxisomal protein .Amino acid alignment analysis revealed two hydrophobic domains. The First hydrophobic domain comprises twenty amino acid residues between 12-31 residues and the second one, is located at 152-169 residues. There is a tripeptide (SKI) at carboxy terminus responsible for sorting of this protein to the matrix of peroxisome. There is a fibronectin type III (FnIII) domain between residues 31-114 in pep. In order to see the importance of above sorting signal, we performed a site-directed mutagenesis to delete SKI tripeptide, FnIII domain and two hydrophobic domains
Materials and Methods: Amplified mutant PEP cDNA were constructed downstream of EGFP cDNA under regulation of CMV promoter in pEGFP-C1 vector and were send for sequence
Results: After transfection of EGFP-PeP/ΔSKI cDNA, cytosolic localization of EGFP fluorescency was observed. Transfection of plasmids containing chimera of EGFP-PEP/Δ31-114, EGFP-PEP/Δ12-31 and EGFP-PEP/Δ152-169 cDNAs into P19 showed several punctuate structures presumably peroxisomes as merged with the pattern of catalase staining in those cells
Conclusion: Taken together, these data strongly suggest that SKI tripeptide located at the C-terminus of protein is essential for peroxisomal targeting

Keywords: Peroxisomal Protein, Site-Directed Mutagenesis, FnIII Domain, Hydrofhobic Domain, SKI Tripeptides

P-54: Construction of Expression Vectors Carrying Mouse Peroxisomal Protein Gene (PeP) with GST and Flag Labels and Transient Transfection of pUcD3/FLAG-PEP Eukaryotic Expression Vector in CHO and P19 Cells

Nazari Jahantigh M[1*], Ghaedi K[2], Nasr Isfahani MH[3], Tanhaei S[3], Rabiee F[3], Karbalaei Kh[3], Nematollahi M[3], Ostad Sharif M[4], Baharvand H[5], Razavi Sh[6]

1. Isfahan University, Biology Departmen, Isfahan, Iran
2. Cell Sciences Research Center, Royan Institite, ACECR, Tehran, Iran
3. Cell Sciences Research Center, Royan Institite (Isfahan Compus), ACECR, Isfahan, Iran
4. Azad University, Genetic Department, Tehran, Iran
5. Cell Sciences Research Center, Royan Institite, ACECR, Tehran, Iran
6. University of Medical Sciences, Anatomical Sciences, Isfahan, Iran

Objective: Peroxisomes are single membrane eukaryotic organelles that perform variable functions. They share a common biogenetic mechanism but have different functions depending on the tissue, the developmental stage or environmental conditions. Peroxisomes are found in all eukaryotic cells and stem cells.Their proteins are encoded by the nuclear genome and imported from the cytoplasm. Peroxisomal matrix proteins are synthesized in the cytoplasm and targeted to peroxisomes by virtue of a peroxisomal targeting signal (PTS). One of the peroxisomal matrix proteins -Peroxisomal Protein (PeP)- has shown different pattern of expression in mouse embryo in various tissues, but the reason is unclear. PeP cDNA was cloned and then subcloned in pGEX6p2 prokaryotic expression vector in order to label this gene with GST to purify PeP protein for further biochemical analysis and identifying related proteins. PeP was inserted downstream of FLAG gene in pUCD3-FLAG eukaryotic expression vector to express tagged-PeP protein for transient transfection analysis and identifying localization of PEP protein.
Materials and Methods: PEP-cDNA was amplified in different PCR reactions using pEGFP-PEP vector and 2 sets of primers introducing specific restriction sites at the ends of PEP. PCR products with BamHI/SalI restriction sites were treated by restriction enzymes and inserted into the pGEX6p2, downstream of GST tag. PEP-cDNA containing BamHI/ApaI restriction sites and FLAG gene -which amplified using pUcD3-FLAG-PEX3 vector- were used as templates in secondary PCR for amplifying FLAG-PEP recombinant DNA. FLAG-PEP fragment was treated by enzymatic digestion and inserted into the pUcD3 eukaryotic expression vector. pGEX6p2-PEP and pUcD3-FLAG-PEP constructed vectors were transformed into the one shot TOP10 and JM105 bacterial competent cells respectively. The positive colonies were selected for plasmid preparation and additional analysis. . Finally, to confirm the intracellular localization of FLAG-PEP, Chinese hamster ovary (CHO) and P19 cells were transfected with the constructed plasmid.
Results: Our results confirmed amplification of the expected products. PEP-cDNA in both PCR reactions encompasses 630bp which encodes 209 amino acid residues. FLAG fragment containing designed sites was 77bp and FLAG-PEP fragment was 700bp. Sequencing of constructed vectors confirms that PEP-cDNA was tagged appropriately and inserted free of mutation and in frame with GST and FLAG. Because of the presence

of SKI in the C-terminal of the related protein, transfection data showed peroxisomal localization of FLAG-PEP as was similar to the catalase. Taken together these data confirmed that PEP is a peroxisomal protein.
Conclusion: We have sub-cloned PEP-cDNA in prokaryotic and eukaryotic expression vectors to tag it with GST and FLAG tandems. Previous studies have indicated that PEP protein is a peroxisomal protein. Transient transfection in CHO and P19 cells with constructed vector confirmed peroxisomal localization of related protein.

Keywords: PEP cDNA, Peroxisome, PEX, pGEX6p2 Vector, PTS1 Signal, pUcD3 Vector

P-55: Isolation and Characterization of Postnatal Stem Cells from Dental Pulp and Priapical Follicle

Navab Azam AR[1*], Fesahat F[2], Sadeghian Nodushan F[2], Soleimani M[3]

1. Dental School Shahid Sadoughi, Oral & Maxillo-facial Surgery, Yazd, Iran
2. Yazd-IVF Center, Stem Cell Lab, Yazd, Iran
3. Yazd-IVF Center, ART Lab, Yazd, Iran
Email: farzanehfesahat@yahoo.com

Objective: Postnatal stem cells have been isolated from a variety of tissues. These stem cells are thought to possess great therapeutic potential for repairing damaged and/or defective tissues. Dental pulp stem cells (DPSCs) and preapical follicle stem cells (PAFSCs) have high proliferative potential for self-renewal has been described and may be important to the regenerative capacity of the tissue. We introduce methodologies for isolation postnatal stem cells from human dental pulp and preapical follicle for the first time in Iran and discuss their potential role in tissue regeneration.
Materials and Methods: In this study postnatal stem cells were isolated from DPSC and PAFSC from patients after local ethical approval and consent letter from the patients. Colony-forming efficiency was assessed with crystal violet. The cells were stained for STRO-1 as a stem cell marker by Immunocytochemistry. To investigate the mesenchymal nature, the adherent cultivated cells were induced to differentiated along osteoblastic and adipogenic lineages.
Results: The isolated cell populations expressed the cell surface molecule STRO-1, a mesenchymal stem cell (MSC) marker. These cells were capable of forming adherent colonies and differentiating into osteocyte and adipocyte.
Conclusion: We have shown that DPSCs and PAFSC are similar to other MSCs and exist in various tissues of the teeth and can use as a source of stem cells for developing bioengineered organs. With this capability in future there is chance of treating periodontal disease and loss of tooth structure either due to pathology or trauma and also it is possible to use stem cell from dental tissue in regeneration teeth and in craniomaxillofacial reconstruction.

Keywords: Mesenchymal Stem Cells, Teeth, Pulp, STRO-1

P-56: Induction of Mesenchymal Stem Cells to Insulin Producing Cells and Evaluation of Their Transplantation in Animal Model of Diabetes

Neshati Z

Ferdowsi University, Biology Department, Mashhad, Iran
Email: neshatizadeh@gmail.com

Objective: Diabetes is the most prevalent degenerative metabolic disease in the world. There are two major types of diabetes: insulin dependent (type 1) and insulin independent diabetes (type 2). Cell therapy is one of the possible approaches for treatment of diabetes. Many researchers have proved the potential of stem cells in treatment of this disease.
Materials and Methods: In present study, mesenchymal stem cells of rat bone marrow were used for in vitro differentiation to insulin producing cells followed by transplantation of these cells into diabetic rats. In vitro differentiation was induced by high glucose concentration, nicotinamide and β-mercaptoethanol and this differentiation was proved by morphological changes and special staining with DTZ (dithizone). Animal model of diabetes was induced by alloxan and differentiated cells were injected into the spleen of diabetic rats. Intra peritoneal glucose tolerance (IPGT) test was done for evaluation of functional improvement of diabetic rats. The one-way ANOVA was done for determination of significant differences.
Results: In vitro differentiated cells were changed from fibroblast-like form to spherical form, and also were crimson red by DTZ. These cells were injected to the diabeic rats. IPGT test showed functional improvement of the test group (transplanted diabetic rats) compared to sham group (untransplanted diabetic rats) after 20 days of transplantation.
Conclusion: Stem cell therapy by in vitro differentiated insulin producing cells can be one of the approaches for treatment of diabetes.

Keywords: Diabetes, Mesenchymal Stem Cell, Cell Transplantation, Insulin Producing Cells

P-57: Epression Patterns of Musashi-1 in Relation to ERapha/PR+Ovarian Cancer Stem Cells

Nikbakht Dastjerdi M

Isfahan University, Department of Anatomy, Isfahan, Iran
Email: nikbakht@med.mui.ac.ir

Objective: The biologic role that estrogen receptor (ER) and progesterone receptor (PR) play in ovarian sex cord-stromal tumors is poorly understood. We in-

vestigated the biology of ERalpha/PR+ cells and their relationship to stem cells in human ovarian cancer.
Materials and Methods: Expression of the putative stem-cell marker Musashi-1 were tested by mmunohistochemistry.
Results: ERalpha/PR+ ovarian cancers exhibit loss of the key regulator of asymmetric cell division, Musashi-1 and thus may arise from symmetric division of the ER-alpha/PR+ stem cell
Conclusion: The data suggest a model in which ER-alpha/PR+ cells are stem cells that self-renew through asymmetric cell division and generate patches of transit amplifying and differentiated cells

Keywords: Cancer Stem Cells, Estrogen, Progesterone, Receptor, Musashi-1

P-58: Pulp and Follicle of Third Molar Teeth: the Unique Accessible Sources of Adult Stem Cells

Niknam M

Shiraz University of Medical Sciences, Shiraz, Iran
Email: dr.mniknam@yahoo.com

Objective: Adult stem cells have been identified in a variety of tissues as a population of multipotential self renewing cells. Third molar teeth in comparison with other sources of adult stem cells such as bone marrow are very accessible and have more stem cells in older ages also these teeth usually are not necessary in dentition and ignore in dental treatment. Goals of this study are isolation, differentiation and characterization of dental pulp stem cells (DPSC) as a unique source of stem cells.
Materials and Methods: After taking an informed written consent, pulps of unerupted third molar teeth achieved through sterile surgery and were dissected and digested by collagenase type I. The obtained single cell supernatant were harvested and cultured. After 6 passages, these cells were prepared for ICC(immune cytochemistry), IF(Imuune fluorescent) and Flowcytometry for the following cell markers: CD73, CD34, CD146, CD45, CD14, CD90, CD19, CD44, CD166, CD105, STRO1, DSPP, Vimentin, keratin, neurofilament and HLA-DR. To evaluate differentiation ability of these cells, cells were incubated in the adipogenic and odontogenic media. When desired morphological changes happened, confirmation of differentiation with histochemical staining, RT-PCR and IF was done.
Results: Stem cells isolated from human third molar dental pulp and follicle display similar features as bone marrow derived mesenchymal stem cells (BM-MSC) do. They are fusiform fibroblast-like plastic adherent cells and are colony-forming. They are smaller in size and have significantly higher proliferation rates compared with BM-MSC. The cell surface markers are in similarity with BM-MSC which are positive for CD73, CD146, CD90, CD44, CD166, CD105, STRO1 and Vimentin and negative for CD34, CD45, CD14, CD19, keratin, neurofilament and HLA-DR. Adipocytes differentiated from DPSC were positively stained with oil red staining and RT-PCR results showed the presence of PPAR-2 (Peroxisome Proliferator-Activated Receptor) gene as an indicator of adipocyte. IF results showed absence DSPP expression before differentiation of odontoblasts and its presence after differentiation as was expected. Alizarin red staining also revealed the presence of hydroxyapatite crystals.
Conclusion: Third molar teeth are very accessible unique tissue resources which contain enough stem cells for isolation, differentiation, clinical application for regenerative dentistry and medicine. DPSCs have benefits over BM-MSCs as they grow faster and have the same differentiation potentials. Although these cells are considered as ectomesenchymal, expression of vimentin on them as a mesenchymal specific marker was an interesting finding in this research raising more evidence as these cells have similar origin with BM-MSCs which is still a controversial issue.

Keywords: Adult Stem Cells, Dental Pulp stem cell, Third Molar Teeth, Odontoblastic Differentiation

P-59: Coculture Investigation of Umbilical Cord Stem Cells from Mouse Embryo with Adult Lung Extract to Study Hematopoiesis

Parivar K[1], Yaghmaie P[1], Kouchesfehani MH[2], Hayati Roudbari N[3*]

1. Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Tarbiat Moallem University, Biology Department, Tehran, Iran
3. Islamic Azad University, Animal Biology Department, Tehran, Iran
Email: nasimhayati@yahoo.com

Objective: Stem cells (SCs) have great therapeutic potential due to their capacity of self-renewal and multilineage differentiation. Umbilical cord cells may be ideal sources of SCs due to their accessibility, painless procedures to donors, promising sources for autologous cell therapy and lower risk of viral contamination. There are many disease related to blood cells disorganized especially white blood cells such as granulocyte and monocytes. Therefore, it is important to find some methods for producing them. GM-CSF (granulocyte-macrophage colony-stimulating factor) in lung extract probably can stimulate hematopoietic SCs to differentiate into granulocyte and monocytes. In this research we used extract of mouse lung that has GM-CSF.
Materials and Methods: To investigate the effects of GM-CSF on stem cells differentiation in vitro, stem cells were isolated from umbilical cord by enzyme digestion and cultured in appropriate growth medium.

In this research four test groups included experimental 1&2 (E1&2) which were exposed to 50% and 70% concentration lung extract for 2 days, respectively, sham (Sh) group which did not exposed to lung extract and control (C) which was same volume of mouse blood that stained immediately. E and Sh groups were incubated for 2 days. All groups were marked with alkaline phosphatase detection kit and giemsa staining methods.
Results: E1&2 cells were differentiated into granulocyte and monocytes. Morphological, histological and differential examinations showed significant changes in E1&2 cells as compared with Sh and C cells.
Conclusion: These findings suggest that GM-CSF in lung extract (especially in E2) has progressive effects on umbilical cord stem cell differentiation into granulocyte and monocyte.

Keywords: Umbilical Cord, Stem Cell, Lung Extract, Differentiation Granulocyte

P-60: Evaluting the Expression of Oct4, Nanog, Sox2 and Ns in Colon Cancer Cell Line (Caco2, HT-29)

Parivar K

Islamic Azad University, Science and Research Brawch, Tehran, Iran
Email: kavan_am@yahoo.com

Objective: Colon carcinoma is the second most common cause of death from cancer.The isolation and characterization of tumorigenic colon cancer cells may help to devise novel diagnostic and theraputic procedures. Uncontrolled self-renewal is recently proposed as an important mechanism in carcinogenesis .Oct4,Nanog,Sox2 and Ns are keyes regulators of pluripotency and self-renewal in embryonic stem cells.The expression of the genes have not been fully studied in somatic cancers,such as colon carcinoma.
Materials and Methods: Here we have investigated the usefulness of Oct4, Sox2, Nanog and Ns gene expression as potential molecular markers in colon cancer cell line caco2 and HT-29.Caco2 and HT-29 human colon cancer cell lines were grown in RPMI medium containing 10%FBS with 1%peniciline and streptomycinen. All cell culture was carried out at 37 in a co2 humidified incubatore.Total RNA was isolated by ISOGEN method. RNA integrity was checked by electrophoresis in agarose gel .RNA concentration was estimated by spectrophotometry at 260 nm.We used RT-PCR to examin samples.The expression of Oct4 and Ns at protein level was further determined by Immunocytochemistry.
Results: OCT4,NANOgSOX2 and NSgene self renewal were expressed in the colon cancer cell line(caco2 &Ht-29).
Conclusion: Collectively,our data approved the expression of Oct4,Nanog,Sox2 and Ns in colon cancer cells and suggested that the expression of these genes can be used as a potential tumor marker for diagnosis and /or prognosis of colon tumors.

Keywords: Cancer Stem Cell, Colon, Self Renewal

P-61: Prostate Stem Cell Antigen Is Overexpressed in Human Transitional Cell Carcinoma

Ranjbaran AR

Tehran Medical University,Tehran Medical University, Tehran, Iran
Email: golnoosh60@yahoo.com

Objective: Prostate stem cell antigen is overexpressed in human transitional cell carcinoma.
Materials and Methods: This is a systematic review article that involved 35 articles about Prostate Stem Cell Antigen Is Overexpressed in Human Transitional Cell Carcinoma(from indexes medicos) of 1985 until 2008 and search of many related topics.
Results: However, despite these efforts, 50% of superficial tumors will continue to recur and as many as 30% will progress to muscle-invasive disease. Although radical cystectomy can salvage many patients with muscle-invasive cancers, a significant number go on to die from metastatic disease, for which there is currently no effective treatment. These data underscore the urgent necessity for better diagnostic and treatment strategies for superficial and invasive bladder cancers. Given the sensitivity of bladder cancer to BCG immunotherapy, there is a particular need to identify bladder cancer antigens for cellular and monoclonal antibody-based targeted immunotherapies. EGFR, for example, is overexpressed by a significant percentage of muscle-invasive bladder cancers. A recent study demonstrated that monoclonal antibody directed against EGFR slowed growth of a human transitional cell cancer in an orthotopic mouse model. Similarly, new bladder cancer markers have been identified that not only demonstrate high specificity for TCC but that also show early promise as a clinical tool.
Conclusion: One such marker, uroplakin II, is an urothelium-specific differentiation antigen that is expressed by; 40% of TCCs. Detection of uroplakin-positive cells in human sera has been associated with metastatic spread of bladder cancer cells and may identify patients with micrometastatic spread prior to undergoing cystectomy. PSCA is a glycosylphosphatidylinositol (GPI) anchored 123- amino-acid glycoprotein related to the Ly-6/Thy-1 family of cell surface antigens. PSCA expression in normal tissues is largely prostate specific, but we recently reported finding PSCA transcripts and protein in transitional epithelium of the bladder and neuroendocrine cells of the stomach. In situ hybridization and IHC analyses demonstrated PSCA expression in more than 80% of local and 100% of bone-metastatic prostate cancer specimens. Importantly, the intensity of PSCA expression increased with tumor grade and stage,

which suggests its potential as an immunotherapeutic target for highrisk and metastatic prostate cancer. Supporting this hypothesis, we recently demonstrated that monoclonal antibodies against PSCA can inhibit tumor growth and metastasis formation and can prolong survival in mice bearing human prostate cancer xenografts. Also, Dannull et al. recently reported that a PSCA-derived peptide could elicit a PSCA-specific T-cell response in a patient with metastatic prostate cancer. Because PSCA is present at low levels in normal bladder, we asked whether PSCA is expressed in TCC. We also determined whether PSCA is overexpressed in bladder cancer compared with normal bladder and whether the level of expression correlates with bladder cancer stage or grade. We demonstrate that PSCA is expressed by amajority of both muscle-invasive and superficial tumors. Moreover, PSCA is overexpressed in virtually all nonmuscle invasive bladder tumors and in .30% of invasive and metastatic cancers. As with prostate cancer, expression increases with increasing tumor grade. Interestingly, the overexpression of PSCA that we observed in TCC is quantitatively more than that seen in prostate cancer with respect to tumor stage. These results support PSCA as a potential diagnostic and/or therapeutic target in bladder cancer.

Keywords: Prostate Stem Cell Antigen, Human Transitional, Cell Carcinoma

P-62: Staurosporine Differentiated Neuronal Cells in Dose Dependent Manner

Sadri S*, Davari Zanjani M, Azadbakht M, Amini A

Razi University, Department of Biology, Kermanshah, Iran
Email: soheil.sadri@gmail.com

Objective: Staurosporine a protein kinase alkaloid inhibitor was recently shown to induce neuronal differentiation in stem cells, neuronal precursor and neuronal cell lines. Staurosporine has paradoxal effects (proapoptotic or neuronal differentiation) depend on concentration. In this study we examined different concentrations of Staurosporine to understand which of them is appropriate for neuronal differentiation without apoptosis induction in PC-12 cells.
Materials and Methods: PC-12 cell line as a useful model system for neurobiological studies was maintained in RPMI 1640 supplemented with 10% FBS.Based on previous studies, Staurosporine were added to culture medium in different concentrations (110, 214, 316 and 1000 nM), then morphology and apoptotic index were assessed by total neurite length estimation and TUNEL staining.
Results: Data showed that total neurite length of PC-12 cells that were treated with 110, 214, 316 and 1000 nM was 44±9.3, 153±15.0, 104±10.4 and 17±9.6μm respectively. Therefore PC-12 cells that were treated with 214 nM Staurosporine had the greatest neurite length in compression with the other concentrations ($p<0.05$). In addition apoptotic index in these concentrations was 4±0.3,4±0.8,8±1.0 and 16±1.1 respectively, Therefore PC-12 cells that were treated with 110 and 214 nM Staurosporine had minimal apoptotic index ($P<0.05$) .
Conclusion: Finally it can be concluded that 214 nM concentration of Staurosporine is an optimum concentration for differentiation of neuronal cell lines to differentiated and mature neuronal cells.

Keywords: Staurosporine, Neuronal Differentiation, Apoptosis, PC-12

P-63: Identification of H2A.Z and H1.0 Histone Variants with Different Expression Patterns During Retinoic Acid Induction of Human Embryonic Carcinoma Cells

Shahhoseini M[1*], Taee A[1], Zare Mehrjardi N[1], Hosseini Salekdeh Gh[1, 2], Baharvand H[3]

1. Department of Stem Cells, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran
2. Physiology and Proteomics Department, Agricultural Biothechnology Research Institute of Iran (ABRII), Karaj, Iran
3. Department of Developmental Biology, University of Science and Culture, Tehran, Iran

Objective: To evaluate the expression of two histone variants, H2A.Z and H1.0, as a epigenetic modification through neural differentiation of human embryonal carcinoma cells, induced by retinoic acid (RA).
Materials and Methods: Human embryonal carcinoma stem cell line (N-Tera2) was induced to neural differentiation by RA treatment, and electrophoretic pattern of histone extracts were compared in different stages, using SDS-PAGE as well as Acid-Urea gel electrophoresis. The observed changes were further analyzed by mass spectrometry and confirmed using western blot.
Results: In addition to dramatic morphological changes, we observed a significant variation in expression pattern of histone H2A.Z after 3 weeks of neural induction, which continues to the end of the culturing time (42 days). For the histone variant H1.0 a narrow upregulation of the protein was observed in the 2nd week of RA treatment, which was further suppressed after one week. It should be noted that these histone variations were parallel to a global changes in acetylation/methylation pattern of histone H3, which are known as key indicators of chromatin changes from stemness to differentiated state.
Conclusion: These results showed the dynamic interplay of histone variants in regulating gene expression during stem cell induction and differentiation.

Keywords: Histone Variant, H2A.Z, H1.0, Neural Differentiation

P-64: Isolation and Primary Culture of Rat Hepatocytes Using Kiwifruit Actinidin Isolation and Primary Culture of Rat Hepatocytes Using Kiwifruit

Actinidin Isolation and Primary Culture of Rat Hepatocytes Using Kiwifruit Actinidin

hirvani Farsani Z

Razi University of Kermanshah, Kermanshah, Iran
Email: ZBShirvani@gmail.com

Objective: Proteolitic enzyme, specially collagenase, are used to digest extracellular matrixe, cells isolation and primary culture. It is important to find new sources of plant or animal protease instead of bacterial or tissue collagenase. In the present research, actinidin, a plentiful protease in kiwifruit (Actinidia deliciosa), was used to isolate rat liver hepatocytes.
Materials and Methods: Rat hepatocytes were isolated by a two-step different doses of actinidin perfusion of liver. isolated hepatocytes were resuspended in Williams, medium E and plated onto plates precoated with rat tail collagen. Isolated cells viability were measured by exclusion of trypan blue and cell morphology were examined by Papanicolauo staining.
Results: Actinidin in concentration of 0.4 mg/ml selectivity isolates Rat hepatocytes by a two-step perfusion method.The separated cells viability was estimated 90-95% in this situation.
Conclusion: The results showed that actinidin has not toxic effect on separated cells and is a novel and suitable protease for isolation of rat liver hepatocytes.

Keywords: Kiwifruit, Actinidin, Protease, Rat hepatocytes, Primary Culture

P-65: Zebrafish Kidney Marrow Contains ABCG2-Dependent Side Population Cells Exhibiting Hematopoietic Stem Cell Properties

Tsinkalovsky O[1*], Laerum OD[1], Fjose A[2], Ferreira S[2], Vik-Mo AO[3]

1. University of Bergen, Pathology, BergenNorway
2. University of Bergen, Molecular Biology, BergenNorway
3. University of Bergen, Center for Medical Genetics, Bergen Norway
Email: oleg.tsinkalovsky@gads.uib.no

Objective: Zebrafish (Danio rerio) has emerged as a powerful genetic model for the study of vertebrate hematopoiesis. However, methods for detection and isolation of hematopoietic stem cells (HSCs) have not yet been reported.
Materials and Methods: In mammals the combination of Hoechst 33342 staining with flow cytometry can be used for separation of a bone marrow side population (SP), which is highly enriched for HSCs. We applied a similar procedure to hematopoietic kidney marrow cells from adult zebrafish, and identified a segregated cohort of SP cells, that demonstrate a set of features typical of stem cells.
Results: SP cells show extremely low scatter characteristics, and are small in size with a minimum of cytoplasm. Treatment of zebrafish kidney marrow cells with reserpine or fumitremorgin C, which inhibit the ABCG2 transporter responsible for Hoechst 33342 efflux, caused a clear reduction in the number of SP cells. Consistent with the quiescent state of HSCs the SP cells are strongly resistant to the myelosuppressive agent 5-fluorouracil. In addition, SP cells specifically demonstrate higher expression of genes known to be markers of HSCs of mammals.
Conclusion: Hence, our results show that the SP phenotype is conserved between mammals and teleosts, and the properties of the zebrafish SP cells indicate a significant enrichment for HSCs. These rapid flow cytometric methods for purification of HSCs from zebrafish may greatly facilitate genetic analysis of stem cells using the advantages of this vertebrate model.

Keywords: Zebrafish, Side Population, Hematopoietic Stem Cells, Flow Cytometry, ABCG2 Transporter

Preccongress Workshops

Hysterosonography Workshop
25 August 2008

With the recent advances in reproductive medicine, hysterosonography has become a safe, simple, and effective outpatient method for evaluation of intrauterine cavity in patients with infertility, abnormal uterine bleeding, recurrent abortion, suspected asherman's syndrome and patients on Tomoxifen therapy. This procedure also known as sonohysterography or saline infusion sonography which is performed using installation of sterile saline in conjunction with transvaginal ultrasound.

Accurate performance and interpreting of hysterosonography images will assist clinicians to minimize diagnostic error. The teamwork between the gynecologist and radiologist is essential, and both should be present at hysterosonography for appropriate evaluation and diagnosis of the problem.

This workshop was organized by Department of Endocrinology and Female Infertility of Royan Institute to provide an overview of the role of hysterosonography as a diagnostic procedure in infertility clinic.

The course included two main parts:

• Detailed lecture including indications, limitations, technique, interpreting of normal and abnormal hysterosonography images.

• Procedure of hysterosonography on the patients with endometrial myoma and polyp, septate uterus, and synechiae.

Target audiences: Gynecologists and Radiologists.

Scientific committee:

- **Tahereh Madani (M.D), Gynecologist**
- **Firoozeh Ahmadi (M.D), Radiologist**

Executive Committee:

- **Fatemeh Zafarani, B.S.c , Midwife**
- **Kiandokht Kiani, M.S.c, Midwife**

Laparoscopy & Tubal Microsurgery Workshop
25-26 August 2008

Laparoscopy has had a remarkable impact in the field of gynecology over a short period of time which is the final diagnostic procedure of any infertility investigation, included in the basic infertility work-up outlined by the American Fertility Society in 1992 and World Health Organization (WHO) guidelines. In addition to pelvic pain Laparoscopy permits evaluation of tubal and peritoneal factors in the infertile patients. A thorough evaluation of the severity of pelvic adhesion or the extent of endometriosis allows selection of the appropriate treatment. Uterine leiomyomas are the most frequent benign neoplasm of the female genital apparatus which is associated with a high morbidity rate, being reported to occur in 20% to 50% of women of reproductive age. The data from several studies show a link between myomas, fetal wastage, and premature delivery. The factors such as the size, number, and location of the tumors influence the choice of the operation. In spite of the increasing improvements in laparoscopic instruments and techniques, advanced laparoscopic technical skills are required to perform laparoscopic myomectomy with success.
Thus the workshop of Laparoscopy and Tubal Microsurgery was held at the Endocrinology and Female Infertility Department of Reproductive Medicine Research Center, Royan Institute (ACECR), Tehran, Iran on Aug 25th and 26th, 2008 (Before the 9th International Royan congress).
The objective of this workshop was specifically focused on developing the knowledge and dexterity of science and technology in laparoscopy and tubal microsurgery and providing the information required by expert surgeons.
The following agenda was established to give to all participants of the workshop:

Aug 25, 2008: Presentation of operative laparoscopic techniques, indications, contraindications and complications given by Dr Sawalhe S, Dr Tehraninejad E
2- Aug 26, 2008,: Presentation of Tubal microsurgery techniques, adhesion prevention, the diagnosis methods of tubal diseases and microsurgery in different part of uterine tube, given by Dr Alborzi S, Dr Tehraninejad E

Target audiences: Obstetric and Gynecology specialists

This activity was sponsored by Parto Rayan Darman Company (representative of Storz's Company in Iran).

Sientific Committee:

- **Samir Sawalhe (M.D), Gynecologist**
- **Saeed Alborzi (M.D), Gynecologist**
- **Ensieh Tehraninejad (MD), Gynecologist**

Executive Committee

- **Nadia Jahangiri (MSc), Midwife**
- **Zahra Ezabadi (MSc), Research Manager**

Paper Evaluation- Peer Review Workshop
26 August 2008

Royan Paper Evaluation- Peer Review workshop was held on August 26th, 2008, beside the Royan International Twin Congress.
After attending this workshop, participants were supposed to know how to prepare a paper suitable for publication in a peer-reviewed academic journal. In particular, it aimed to aware the participants of the scientific and presentational standards required to satisfy referees and editors during the paper evaluation process.

Main subjects of the workshop included:
- How to write the perfect scientific paper?
- What should be in the results section of a scientific paper?
- The importance of statistics. How to impress the editor
- Dealing with Plagiarism & Fraud
- Key issues in clinical trials
- Interactive tutorial sessions: the participants worked with faculty members in small groups. Published articles were evaluated. Strengths and weaknesses were assessed within the groups and later reported to all participants.

  Group 1: Titles & Abstracts. Group 3: Materials & Methods
  Group 2: Figures & Tables Group 4: Results & Discussion

- Feedback and concluding discussion

Scientific Committee:
- **Prof. Stephen Hillier (UK)**
- **Dr. Babak Eshrati (Iran)**
- **Prof. Helen Mardon (UK)**
- **Prof. Thomas Ebner (Austria)**
- **Dr. Behrooz Astaneh**
- **Dr. Ali Shaidzadeh**
- **Ms. Houri Mivechi (Iran)**
- **Dr. Saeid Abroun (Iran)**
- **Dr. Reza Omani Samani (Iran)**

Executive Committee:
- **Ms. Laila Daliri**
- **Dr. Azadeh Oraei**
- **Ms. Leila Alizadeh**
- **Ms. Farideh Malekzadeh**

Preimplantation Genetic Diagnosis (PGD) Workshop
25-26 August 2008

Preimplantation Genetic Diagnosis (PGD) has become an essential part of preventive measures for genetic disorders, enabling couples to avoid termination of pregnancy after routine prenatal diagnosis. It allows the detection of errors in early preimplantaion development and prediction of the genotype of the resulting embryo prior to implantation, so that only normal embryos and those with optimal developmental potential are preselected for transfer to the uterus, ensuring an unaffected pregnancy and the birth of a healthy baby. In this way, couples at high risk of having offspring with genetic disease have an option to control the outcome of their pregnancy from the outset.

PGD is performed through PB or blastomere biopsy, which has no deleterious effects on the pre- and postimplantation development. The present PGD experience includes approximately 2000 clinical cycles performed for single-gene defects, with the majority of cycles resulting in embryo transfer, and more than one-quarter in clinical pregnancy and the birth of unaffected and apparently healthy children. Therefore, PGD for single-gene disorders may be considered to be a safe, accurate and reliable technique, with growing value for prevention of genetic disorders.
Available experience provides the basis for the wider application of PGD for any genetic disease currently diagnosed by prenatal diagnosis, and also indications for prenatal diagnosis has not previously been practiced, such as late-onset and complex disorders, congenital malformations, blood group incompatibility and preselection of unaffected and HLA-matched embryos. This extends the practical value of PGD, with its utility being no longer limited to the prevention of single-gene disorders, by expanded it to the treatment of siblings requiring stem cell transplantations.
The 2-day hands-on-work Royan international workshop on molecular Preimplantation Genetic Diagnosis was the finest course in the subject. The workshop consisted of a series of lectures, demonstrations and laboratory practices that covered various aspects of molecular diagnosis of single gene disorders. The program was provided to established investigators, scientists with prior experience in molecular biology and medical genetics, and experts in the field of ART technology and medical genetics.

Scientific Committee:

- **Prof. Benkhalifa Moncef (France)Nasab**
- **Dr. Hamid Gourabi (Iran)**
- **Dr. Mojtaba Rezazadeh (Iran)**
- **Dr. Poopak Eftekhari**
- **Mr. Anil Biricik**
- **Ms. Leily Karimian**

Executive Manager:

- **Mr. Hamed Vaziri**

Vitrification Workshop
26 August 2008

Cryopreservation of reproductive entities is gaining a constantly growing importance. What used to be a means for storage of semen and embryos now has a much wider application. On top of being part of ongoing subfertility treatment, it now also plays an important role in extension and preservation of fertility.
We see an increase in cumulative pregnancy rate, a possibility of single embryo transfer, a less troublesome second treatment, a possibility to deal with legal restrictions (e.g. Italy), and a number of practical issues.
We have the possibility of extending and preserving fertility of health reasons, e.g. cancer and premature menopause, and of social and personal reasons varying from country to country.
With more or less success, the embryologists are now able to preserve all types of reproductive structures – from gonadal tissue and immature oocytes to hatched blastocysts. Still there are only two main methods – slow freezing and vitrification.
Royan Vitrification Workshop was held on August 26th, in cooperation with Medicult Company. This workshop and was aimed to touch on slow freezing, but mainly looked at various aspects of vitrification: What is vitrification, which tools are used, how is it done, and how successful is it?

Scientific Committee:

- **Prof. H. Ingolf Nielsen (Denmark)**
- **Ms. Leily Karimian (Iran)**

Executive Members:

- **Dr. Poopak Eftekhari Yazdi**
- **Ms. Fatemeh Hasani**

Zona-Free Somatic Cell Nuclear Transfer Workshop
25-26 August 2008

Introduction:

Cloning by nuclear transfer from adult somatic cells (SCNT) is a remarkable demonstration of the developmental plasticity. When a nucleus is placed into oocyte cytoplasm, the changes in chromatin structure that govern differentiation can be reversed, and the nucleus can be reprogrammed to support development to term. However, the widespread application of SCNT technology has been hampered by biological and technical quandaries. Recently introduced zona-free procedures have offered a solution for the latter problem. The most radical approach of these techniques is the so-called **Zona-Free SCNT** which is a rapid and efficient technique that suits large-scale NT programs. It requires less expertise and time than traditional NT methods. Production efficiency is high and embryo quality, in terms of pregnancy rates and live births, is great.

Following our successful International SCNT Workshop in 2006, the organizing committee decided to hold **The First International Workshop on Zona Free SCNT** on August 25-26, 2008, which took place in Isfahan campus of Royan Institute, in conjunction with the Royan International Twin Congress (August 27-29, 2008). The workshop aimed to bring together researchers from both the academic and research centers working, or wishing to work, on SCNT. The Workshop was held by Associated Prof. M.H. Nasr-Esfahani and Prof. Bijorn Oback, the senior scientist at the Agriculture Research Institute (NZ) who assisted the theoretical and practical aspects of his novel zona-free SCNT technique.

Main topics of the Workshop included:

Oocyte maturation
Zona Pellucida removal
Enucleation
Attachment of donor cells to the zona free-oocytes
Electrofusion and activation
In vitro culture

Scientific Board:

- **Prof. M.H. Nasr Esfahani (Iran)**
- **Prof. Bjorn J. Oback (New Zealand)**

Executive managers:

- **Ms. Azam Dalman**
- **Dr. Seyed Morteza Hosseini**

Authors Index

The simulation or the conformation ?

LS used drugs have a code to identify...

yet Cyclogest® conforms

Cyclogest®

Bio-identical progesterone *Micronized Crystals<10 microns*

- **Identically matches the somatic progesterone molecule & its expected actions**
- **Dual administration routes; vaginal and rectal conforms with the human anatomy**
- **Unique vehicle;**
  - Self-lubricating properties rendering the application neither painful nor messy
  - Controlled slow release of the active hormonal ingredient for easier once or twice daily dose

When to use Cyclogest® ?

Whenever progesterone supplementation is needed up to the specialist's experience...

- **Luteal phase support during IVF techniques**
- **Recurrent miscarriages (RPL) attributed to luteal phase deficiency**
- **Labeled for the following indications:**
  - puerperal depression
  - premenstrual syndrome, including premenstrual tension and depression

actavis
creating value in pharmaceuticals

Further information is available upon request
Actavis M. East & Africa Regional Office
P.O.Box 13469 Dubai, U.A.E.
E-mail : actavis@actavis.com
www.actavis.com

ACTOVERCO
Together for a healthy future

شرکت اکتوورکو
نمایندگی و دفتر علمی کمپانی اکتاویس در ایران
تهران، سعادت آباد، خیابان دشت بهشت، پلاک ۱۷
تلفن: ۲۲۰۶۸۴۷۹(۰۲۱) فاکس: ۲۲۰۶۱۷۰۴(۰۲۱)

اویترل OVITRELLE®

۲۵۰ میکروگرم در ۰/۵ میلی لیتر
محلول جهت تزریق در سرنگ از قبل پرشده
کوریوگنادوتروپین آلفا

اویترل فرآورده ای محتوی کوریوگنادوتروپین آلفا می‌باشد که بسیار شبیه گنادوتروپین جفتی است که به طور طبیعی در انسان یافت می شود، ولی در آزمایشگاه و توسط تکنیک خاص DNA ی نوترکیب تولید می شود. این دارو در دسته ای از هورمونها بنام گنادوتروپینها جای دارد که در کنترل فرآیند تولیدمثل دخیل می‌باشند. اویترل درزنان تحت درمان با تکنیکهای کمک باروری مانند لقاح در آزمایشگاه(IVF) استفاده می شود. سایر داروها در ابتدا جهت رشد و نمو فولیکولهای متعدد وتولید تخمک تجویز می‌شوند. سپس با مصرف اویترل این فولیکولها بالغ و رسیده می‌شوند. همچنین اویترل در زنانی که فاقد تخمک گذاری (anovulation) هستند یا افرادی که تخمکهای اندکی تولید می‌کنند (oligo-ovulation)، مصرف می‌شود. بعد از مصرف سایر داروهایی که رشد فولیکولها را موجب شده اند، این دارو سبب آزاد شدن تخمکها (اوولاسیون) می‌شود. قبل ازشروع درمان وضعیت باروری بیمار و همسر اوباید بررسی شود.

اویترل نباید مصرف شود:

- در بارداری و یا شیر دهی
- بزرگ شدگی تخمدان و یا یک یا چند کیست بزرگ تخمدانی
- خونریزیهای واژینال با منشاء ناشناخته
- سرطان تخمدان، رحم یا پستان
- اگر توموری در غده هیپوتالاموس یا هیپوفیز وجود داشته باشد
- التهاب شدید عروق یا لخته در عروق (اختلالات فعال ترومبو- آمبولیک)
- اگر بیمار در ۳ ماه اخیر حاملگی خارج رحمی داشته است
- در شرایطی که حاملگی نرمال را ناممکن می سازد (فقدان رحم، تخمدانی که رشد لازم را نداشته، فیبروئید) یا درحین یائسگی
- سابقه آلرژی (افزایش حساسیت) به کوریوگنادوتروپین یا مواد جانبی اویترل

احتیاط ویژه با اویترل:

درمان با اویترل ممکن است خطر ابتلا به سندرم بیش تحریکی تخمدان (OHSS) را افزایش دهد (عوارض جانبی احتمالی را ببینید). این زمانی است که تخمدانها بیش از حد به درمان پاسخ می‌دهند و تعداد زیادی فولیکول رشد می‌کنند. شایعترین علامت آن درد شکمی است. اگر بیمار به درد شکمی آشکاری مبتلا شد یا احساس ناراحتی کرد، تزریق دیگری انجام نداده و در اسرع وقت با پزشک یا پرستار مشورت نماید. همچنین باید به مدت حداقل چهار روز از مقاربت جنسی پرهیز نموده و یا از روشهای جلوگیری مکانیکی (کاندوم) استفاده شود. در مقایسه با بارداری طبیعی، احتمال چند قلوزایی با این درمان افزایش می‌یابد. شایعترین آن دو قلو زایی است. در تکنیکهای کمک باروری، تعدد فرزندان به تعداد جنین های جایگزین شده بستگی دارد. خطر ایجاد (OHSS) یا چندقلوزایی با مصرف صحیح اویترل و پایش دقیق و منظم (تستهای خونی جهت سنجش استرادیول و اولتراسوند) درطول دوره درمان کاهش می‌یابد.

استفاده از داروهای دیگر: در صورتی که بیمار از داروهای دیگری استفاده می‌کند یا اخیراً مصرف کرده است، که داروهای قابل تجویز بدون نسخه را نیز شامل می‌شود، باید پزشک یا داروساز خود را مطلع نماید.

بارداری و شیردهی: در بارداری و یا شیر دهی اویترل نباید مصرف شود.

نحوه استفاده از اویترل:

اویترل باید دقیقا مطابق دستور پزشک مصرف شود. دوز معمول اویترل به صورت یک تزریق ازسرنگ آماده (۲۵۰ میکروگرم در ۰/۵ میلی لیتر) می‌باشد. پزشک باید در مورد زمان دقیق مصرف دارو به بیمار توضیح دهد.

اویترل جهت تجویز زیر جلدی درنظر گرفته شده است، به این معنی که در زیر پوست تزریق می‌شود. هر سرنگ فقط برای یکبار مصرف است. فقط محلول شفاف و بدون ذره باید استفاده شود. گاهی تزریق توسط پزشک یا پرستار انجام می‌شود و یا ممکن است بیمار و یا همسراو جهت انجام تزریق در منزل آموزش ببینند.

دستورالعمل نحوه تزریق:

۱ـ دستها باید شسته شود. مهم است که دستها و وسایلی که استفاده می شود تا حد امکان تمیز باشند.

۲ـ پنبه الکلی در بسته موجود نمی باشد. همه وسایل را روی سطح تمیزی باید قرار داد (دو عدد پنبه الکلی، یک سرنگ از قبل پرشده حاوی ماده دارویی.)

۳ـ تزریق: محلول باید فوراً تزریق شود: پزشک یا پرستار در مورد محل تزریق راهنمایی می‌کنند (برای مثال شکم، جلوی ران.)

محل انتخابی باید با پنبه الکلی تمیز شود. پوست را محکم به طرف بالا کشیده و سوزن را با زاویه ۴۵ تا ۹۰ درجه با یک حرکت سریع وارد پوست کنید. دارو را زیر پوست تزریق کنید. نباید دارو بطور مستقیم وارد ورید شود. محلول را با فشار آهسته تزریق کنید. تمام محلول را هر قدر که بطول می انجامد، تزریق کنید، سپس سوزن را فوراً خارج کرده و با یک پنبه الکل با حرکت دورانی پوست را تمیز کنید.

۴ـ کلیه وسایل استفاده شده را دور بریزید: به محض اینکه تزریق انجام شد، فوراً سرنگ خالی را در ظرف مخصوص مواد زائد نوک تیز بیاندازید. هر اندازه از محلول که استفاده نشد، نیز باید دور ریخته شود.

در صورتیکه اویترل بیش از حد مصرف شود:

اگر اویترل بیش از حد مصرف شود، ممکن است سندرم تحریک بیش از حد تخمدان رخ دهد که در قسمت احتیاط ویژه و عوارض جانبی احتمالی بدان اشاره شده است. در صورت مشاهده علائمی از سندرم باید با پزشک مشورت شود.

در صورت فراموش شدن مصرف اویترل:

باید با پزشک تماس گرفته شود.

عوارض جانبی احتمالی

اویترل هم مانند سایر داروها می‌تواند سبب بروز عوارض جانبی شود، اگرچه همه افراد دچار عارضه نمی شوند. بیشتر عوارضی که تابحال مشاهده شده اند، خفیف تا متوسط بوده اند. شایعترین عوارض جانبی که گزارش شده اند شامل خستگی، درد و واکنش موضعی در محل تزریق می‌باشد. در مطالعات بالینی سندرم تحریک بیش از حد تخمدان تقریباً در ۴ ٪ بیماران مشاهده شده است، که بیشتر این موارد خفیف تا متوسط بوده اند. تجمع خون در حفره پریتونئال و مشکلات تنفسی از عوارض احتمالی سندرم تحریک بیش از حد تخمدان می‌باشند. این سندرم بوسیله کیستهای بزرگ تخمدان متمایز می‌شود. اولین علامت بیش تحریکی تخمدان، درد در قسمت تحتانی شکم است که ممکن است با تهوع، استفراغ و افزایش وزن همراه شود. در صورت مشاهده علائم فوق الذکر باید در اسرع وقت آزمایشات پزشکی انجام شود. شایعترین عوارض گزارش شده شامل درد شکمی، تهوع و استفراغ، سردرد، التهاب و واکنش در محل تزریق و سرگیجه می‌باشد. در برخی از موارد اسهال، افسردگی، تندخویی، بیقراری و درد پستان گزارش شده اند که غیرمعمولتر می‌باشند. احتمال حاملگی خارج رحمی، پیچش تخمدان (شرایطی که تخمدانها را تحت تاثیر قرار می‌دهد) وبروز سایر عوارض ممکن است توسط تکنیکهای کمک باروری که پزشک استفاده می‌نماید، افزایش یابد. موارد محدودی از واکنشهای آلرژیک به اویترل (راش) گزارش شده اند.

نگهداری اویترل

دور از دسترس اطفال نگه داشته شود. بعد از گذشتن تاریخ مصرف ذکر شده، ازدارو استفاده نشود. تاریخ انقضا مربوط به آخرین روز ماه می‌باشد. در یخچال (دمای ۸-۲ درجه) و بسته بندی اصلی نگهداری شود. محلول تزریقی اویترل ۲۵۰ میکروگرمی ممکن است در دمای اتاق (۲۵ درجه یا کمتر) تا ۳۰ روز نگهداری شود. اگر در این مدت به یخچال برگردانده نشده و مصرف هم نشود، باید دور انداخته شود. اویترل جهت یکبار مصرف است و هر محلول استفاده نشده‌ای باید دور انداخته شود.

اطلاعات تکمیلی:

اویترل حاوی: ماده موثره آن کوریوگنادوتروپین آلفا می‌باشد. هر سرنگ از قبل پر شده حاوی ۲۵۰ میکروگرم در ۰/۵ میلی لیتر است (معادل ۶۵۰۰ IU)، سایر مواد جانبی شامل مانیتول، متیونین، پلاکسامر ۱۸۸، اسید فسفریک رقیق شده، هیدروکسید سدیم، آب برای تزریق می‌باشد.

شکل ظاهری اویترل و محتویات بسته: اویترل بصورت محلول قابل تزریق تهیه شده است. این دارودرسرنگ پرشده و در بسته های یک عددی در دسترس می‌باشد.

این برگه راهنما در ژانویه ۲۰۰۶ بازنگری و تائید شده است.

تولیدکننده شرکت داروسازی Merck Serono:
Industria Farmaceutica Serono S.p.A , Zona Industriale di Modugno, I-70123 Bari, Italy

وارد کننده: شرکت کمال دارو نمایندگی رسمی شرکت Merck Serono در ایران

توزیع کننده: شرکت پخش رازی

جهت دریافت اطلاعات بیشتر با آدرس الکترونیکی info@kamaldarou.com شرکت کمال دارو تماس بگیرید

OVITRELLE®
choriogonadotropin alfa

hCG ONE CHANCE. MAKE SURE YOUR PATIENTS GET IT RIGHT.

Merck Serono | *Leading Innovation In Fertility*

MERC
SERO

Decapeptyl 0.1®

TRIPTORELIN

For a successful pituitary down-regulation

1. **Accurate measured dose.**
   (No dose adjustment is needed).
2. **Simple dose administration.**
   (Ready to use, no need for preparation).
3. **Minimum dose loss and any risk of contamination.**
   (Ready to use, no need for preparation).
4. **Patients' convenience.**
   (Once daily administration).

FERRING
PHARMACEUTICALS

Further information is available at:
Ferring Middle East, PO BOX 850845, Amman 11185, Jordan
Tel: ++962 5501000, Fax: ++962 6 5501015
e-mail: support@ferring.com.jo
Visit our website at : www.ferringme.com

GEA Process Equipment Division

Niro Soavi

ایران پنام (با مسئولیت محدود)

۱- NEW BRUNSWICK SCIENTIFIC معتبرترین سازنده انواع شیکرها، انکوباتورهای CO2 ، سیستم های تهیه و توزیع محیط کشت، فریزرهای ۸۶- درجه سانتیگراد، فرمانتورها و بیوراکتورها

۲- PLANER انگلستان پیشرو در ساخت سیستم های Cryopreservation شامل فریزرهای قابل برنامه ریزی جهت IVF ، بانک اعضا و سلول

۳- RESEARCH INSTRUMENTS انگلستان، معروفترین سازنده انواع سیستم های میکروواینجکشن و لیزرهچینگ جهت بیوپسی جنین

۴- HWL SCIENTIFIC از کمپانی Active Vibration System آلمان به منظور حذف لرزش های احتمالی موجود در محیط و افزایش دقت عمل میکروواینجکشن

۵- STATEBOURNE انگلستان و MVE امریکا، سازنده تانک های حمل و نگهداری ازت مایع

۶- GONOTEC آلمان، ارائه کننده سیستم های اسمومتر شامل Osmomat 030 و Osmomat auto مورد استفاده در مراکز پزشکی ، تحقیقاتی و IVF

۷- MEDITE آلمان، سازنده پیشرفته ترین تجهیزات پاتولوژی، سیتولوژی و هیستولوژی

۸- MSE انگلستان، سازنده انواع سانتریفوژهای معمولی و یخچال دار

۹- ROCKET MEDICAL انگلستان، سازنده انواع اینسترومنتهای جراحی و انواع کاتاترها

۱۰- SCIENTIFIC LABORATORY SUPPLIES انگلستان، عرضه کننده انواع سیستم ها، لوازم و مواد مصرفی برای تمامی آزمایشگاه ها

۱۱- NIRO SOAVI ایتالیا، عرضه کننده انواع هموژنایزرهای آزمایشگاهی و صنعتی

۱۲- LABPLANT انگلستان، تولید کننده دستگاه های اسپری درایر (مولد پودر)

۱۳- ERLAB فرانسه ، معتبرترین سازنده انواع هودهای آزمایشگاهی

۱۴- AQS انگلستان، پیشتاز در تولید تجهیزات آزمایشگاهی متنوع نظیر بن ماری شیکر، ترازو، میکرو سانتریفوژ و غیره

۱۵- BIOTEC_ FISCHER آلمان ، عرضه کننده انواع الکتروفورز های عمودی و افقی مورد استفاده در مراکز پزشکی و تحقیقاتی

۱۶- ASTELL انگلستان، عرضه کننده انواع اتوکلاوها در حجم های مختلف

۱۷- EXPLERA ایتالیا عرضه کننده انواع سیستم های بیولوژی ملکولی ، معرف ها و کیت ها شامل دستگاه های PCR ، Gel Documentation و ترانس لومیناتور

۱۸- ARTmoderne Labortechnik آلمان، پیشتاز در جهت ارائه انواع هموژنایزرهای کوچک آزمایشگاهی جهت هموژن کردن ترکیبات گوناگون در حداقل زمان ممکن

آدرس : تهران ، خیابان کریم خان زند ، بین ایرانشهر و جردمند جنوبی ، ساختمان ۱۱۶ ، طبقه ۳ ، واحد ۱۲ صندوق پستی: ۱۵۸۵۵/۱۳۱

تلفن : ۸۸۸۴۱۸۸۰ و ۴ - ۸۸۳۲۱۰۷۰ فاکس : ۸۸۳۲۱۰۶۹

Email : panam@rasaneh.com

Web Site: wwwiran-panam.com

Fostimon® hFSH

Highly Purified hFSH

Merional® hMG

Highly Purified hMG

Choriomon® HCG

Highly Purified hCG

A new vision in medicine

KARL STORZ FULL HD

High Definition – more precision for endoscopic images

KARL STORZ – FULL HD platform

In medicine, precision and focus are key terms. In order to continue developing and enhancing quality standards KARL STORZ has developed a new tool – the FULL HD platform – which guarantees the user a new visual dimension.

The highest resolution possible – 5 times greater than the standard – combined with the16:9 format guarantees brilliant picture quality and larger display details.

Now, with an upgrade of the IMAGE1™ CCU, HD quality is becoming ever more accessible to the field of medicine: from the HD camera head right up to the HD WideView™ monitor.

All this is perfectly in line with the KARL STORZ philosophy that all previous IMAGE1™ systems as well as their numerous functions are fully compatible and can therefore be used with the FULL HD platform too.

STORZ
KARL STORZ — ENDOSKOPE

THE DIAMOND STANDARD

KARL STORZ GmbH & Co. KG, Mittelstraße 8, 78532 Tuttlingen/Germany
Telephone: +49 (0)7461 708-0, Fax: + 49 (0)7461 708-105, E-Mail: info@karlstorz.de, Web: www.karlstorz.com
KARL STORZ Endoskope, Regional Center for Endoscopy (Offshore) s. a. l., St. Charles City Center, 5th Floor, Phoenicia Street, Mina Elhosn, 2020 0908 – Beirut, Lebanon,
Telephone +961 (1) 368181, Fax: +961 (1) 365151, E-mail : info@karlstorz-rce.com, Web : www.karlstorz.com

PRDE
Parto Rayan Darman Engineering Co.
No. 460 - Day Alley - 6th St. - Iran Zamin Ave.Shahrak Gharb - Tehran - Iran
Tel.(+98)21- 88368183-90 , Fax (+98)21- 88083537 www.PRDE.net e-mail : info@prde.net

ARYAN RAYKA Co. Ltd.

Lab & Med Products

Systems,Instruments,Equipment

- Laser Hatching System
- Sperm Analyzers
- Micromanipulation
- Cryopreservation
- Freezing Systems
- Liquid Nitrogen Containers
- Heating Stages for Microscopes
- Warming Plates

No.91 - Jalale Ale Ahmad Exp. Way-Tehran 14466-Iran
Tel : +9821 88243460, Fax: +9821 88243459
Email : aryan-rayka@saed-info.net

Nekou Azma Teb Co.

supplier & distributor of equipment Laboratory
research biochemical , molecular biology , genetics

Dr.Seyed Mohsen Nekounazar azad
Doctor of medical technology
Managing director & chairman

Tel: 22081099-22081113, Telefax: 220811098, Mobile: 09121271721
Email: Drmnazad@yahoo.com

SAKAN TEB Co. (P.J.S)

شرکت سکن طب (سهامی خاص)

نماینده انحصاری کمپانی‌های

Vitrolife
Innovative Cell and Tissue Technology

- Vitrolife سوئد، تولیدکننده محیط‌های کشت GIII Series و Embryo Glue و محیط کشت Stem cell، ICSI (PVP) و Goil

SWEMED

- SWEMED سوئد، تولیدکننده سوزن‌های پانکچر، آمنیوسنتز، بیوپسی جفت، کاتتر ترانسفر، سوزن P.O.B دارای تاییدیهٔ FDA آمریکا،

NARISHIGE JAPAN

- Narishige ژاپن، سیستم‌های میکرواینجکشن

TOKAI HIT

- Tokaii Hit ژاپن، ترموپلیت‌های مخصوص کلیهٔ میکروسکوپ‌ها

Fornax

- Fornax هندوستان، سانتریوفوژهای ترموپلیت‌دار، گرم کننده‌های لوله‌های انکوباسیون، تهویهٔ هوای آزمایشگاه IVF و بلوک‌های گرم‌کنندهٔ ظروف

Cryo Bio System
Groupe I.M.V. Technologies

- Cryobiosystem فرانسه، تولیدکنندهٔ دستگاه‌های بسته‌بندی High Security Straws

درس:
هران، خیابان دکتر شریعتی، بعد از پل آیت‌الله صدر،
وچه اخوان، پلاک ۲۸، طبقه اول کدپستی: ۱۹۳۱۶
لفن: ۲۲۲۳۳۱۳۳ - ۸۸۷۶۳۶۸۱ - ۲۲۶۷۷۵۶۰
فکس: ۲۲۱۴۷۷۳

E-mail: shokoohi@yahoo.com sakanteb@hotmail.com

wallace

for this generation and the next

We were at the birth of IVF

Wallace worked with the pioneers of IVF treatment.

Since then thousands of babies have been born with the help of Wallace products.

What will yours?

Ask your consultant about **Wallace**

We're still growing with it

Innovation in Critical and Community Care

JARF KHERAD Co.

Tel: (+9821)88100861
Fax: (+9821)88100862

SMITHS INDUSTRIE

Medical Systems

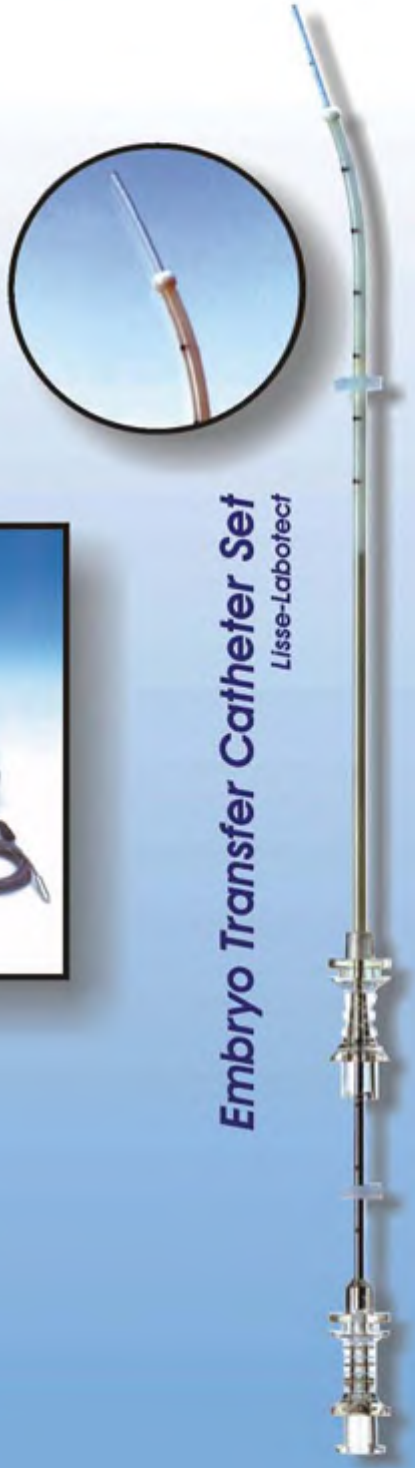
Labotect

Labor-Technik-Göttingen

Thereby :better recovery time

InControl 1050
measuring ,calibrating
& recording
CO_2 and temp,
in CO_2-incubators

Incubator C200 with 6 small doors



Embryo Transfer Catheter Set
Lisse-Labotect

فنون آزمایشگاهی
LAB TECH

Lab Tech (Fonoon Azmayeshgahi)Ltd.
No. 30,Baseghi Corner,Kavoosifar St., Sh. Beheshti Ave.,
Tehran-15779, IRAN. P.O.Box:15875-4961,
TEL.:+98 21 88 74 80 00, Fax:+98 21 8874 80 10
E.Mail:info@fonoon.co.ir

OLYMPUS

Official Distributor

فنون آزمایشگاهی
LAB TECH

Lab Tech (Fonoon Azmayeshgahi)Ltd.
No. 30,Baseghi Corner,Kavoosifar St., Sh. Beheshti Ave.,
Tehran-15779, IRAN. P.O.Box:15875-4961,
TEL.:+98 21 88 74 80 00, Fax:+98 21 88 74 80 10
E.Mail:info@fonoon.co.ir